

Forensically Important Blow Flies (Diptera: Calliphoridae) of Iran with two new records for the country

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Abstract

Blowfly (Diptera: Calliphoridae) identification is a pre-requisite to the field of sanitary biology, medical, veterinary, and forensic entomology. The specimens were collected using fly traps in eight regions of Fars province, Iran. A total of 10 species belonging to four genera were recorded out of which *Calliphora pattoni* Aubertin, 1931 and *Chrysomya phaonis* (Seguy, 1928) are new records for the country.

Keywords: *Calliphoridae, check list, Blowfly, Fars province, Iran.*

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Introduction

The members of Calliphoridae (Diptera: Oestroidea) represent cosmopolitan group of calypterate flies, widely distributed in all the zoogeographical regions of the world. The group is represented by 1500 species included in about 150 genera from the world (Triplehorn and Johnson, 2005; Tomberlin *et al.*, 2011). This heterogenous group of flies is of great significance in terms of medical, veterinary, and economic concerns. Some of the species belonging to subfamily Calliphorinae, Chrysomyinae, Luciliinae and Toxotarsinae are well known for their potential to calculate post-mortem interval (minPMI) in homicidal cases, parasitizing animal tissue and for transmission of pathogenic microorganisms (Hall & Wall, 1995; Rognes, 1991; Hassan *et al.*, 2018). Adults of Calliphoridae are medium to large sized flies, often metallic blue, or metallic green in colour. These are among the first insects to arrive at corpse and the larvae play a significant role in decomposition of the carrion (Byrd & Castner, 2009). Because of their ubiquitous presence, forensic entomologists strongly rely on blow flies as substantial evidence, and its reliability depends on correct species identification of the collected insect evidence

from the crime scene (Akbarzadeh *et al.*, 2015). Herein, we present a checklist of forensically important blow flies in Fars Province, with new records for the country.

Materials and Methods

The study was conducted in the months of June to October for two consecutive years (2015 and 2016). The samples were collected from different parts of Fars province (27°2'-32°42'N, 50°42'-55°38'E), which include Shiraz, Marvdasht and Sivand in North, Kuhmare Sorkhi in South, Maharlu in Southeast, Beyza, Ghalat and Ardakan in Northwest (Fig.1). For collection of blow fly specimens, pyramidal traps and plastic water bottle fly traps were employed. A dog carcass was also used as bait to collect maximum diversity of forensically important flies from the region. The corpse was placed in a metal frame cage (1.5m²) covered with mesh wire screen, to protect the carcass from stray animals and vertebrate scavengers. The blow fly specimens thus collected were identified following the descriptions as detailed in Senior-White *et al.* (1940); Akbarzadeh *et al.* (2015).

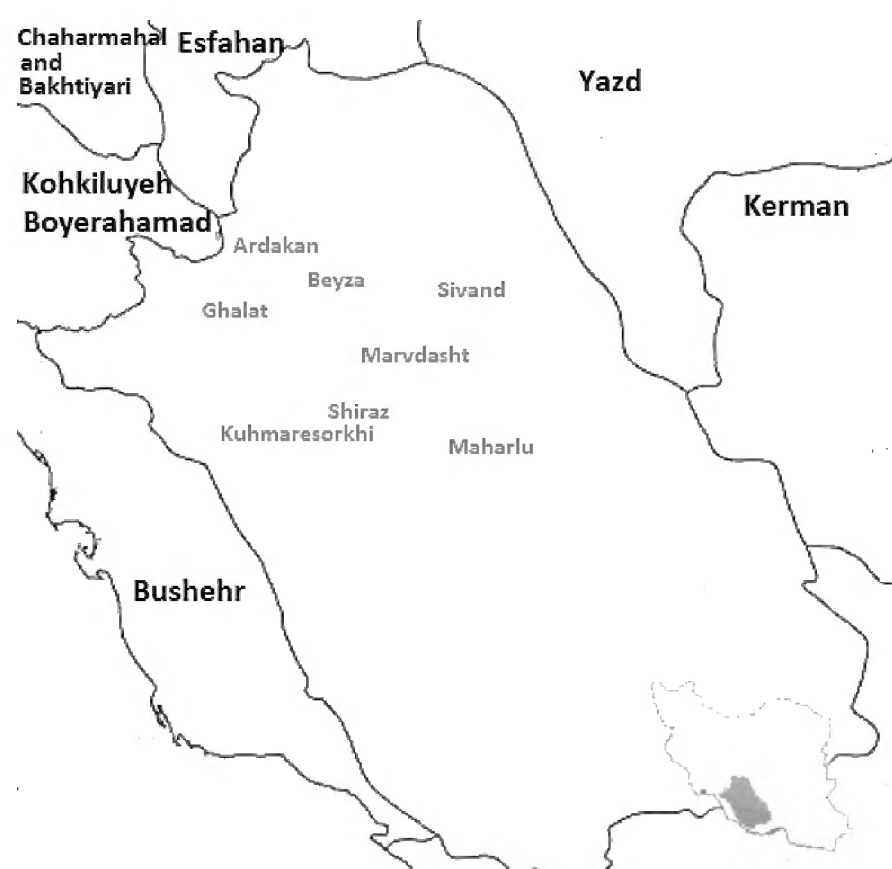


Figure 1: Blow fly collection localities in Fars Province, Iran

Results

A total of 18677 calliphorid specimens were collected from the study area, falling in four subfamilies: Calliphorinae, Luciliinae, Chrysomyinae, and Polleniinae. A total of 10 species were collected of which *Calliphora pattoni* and *Chrysomya phaonis* are new records for the country (Table 1).

Discussion

Blowflies occur in all biogeographic regions of the world (Thompson, 2006) and have been extensively studied in South America (Carvalho and Mello-Patiu, 2008), Argentina (Mariluis *et al.*, 2008), North America (Whitworth, 2006; Marshal *et al.*, 2011), Portugal (Prado e Castro *et al.*, 2016), East and South Asia (Bharti, 2011; Yang *et al.*, 2014; Hassan *et al.*, 2018), and Afrotropical region (Irish *et al.*, 2014). Blow flies of the middle East region were studied by Akbarzadeh *et al.* (2015) and a key was provided. The present study was conducted in the Fars Province of Iran (one of the 31 provinces) located in the southern part of the country. Due to the topography of the studied region, the area witnesses distinct climatic seasons and thus support the development of varied blow fly species. Akbarzadeh *et al.* (2015) and Keshavarzi *et al.* (2016) reported the presence of *Calliphora vicina*, *Lucilia sericata*, *Chrysomya albiceps*, *Chrysomya bezziana*, *Chrysomya rufifacies* from the region. The present study has added *Pollenia rudis* as new record for the province and *Calliphora pattoni* and *Chrysomya phaonis* as new records for the country.

Table 1: List of blow fly species collected from Fars Province region of Iran

Family	Subfamily	Genus	Species
Calliphoridae	Calliphorinae	<i>Calliphora</i>	<i>Calliphora vicina</i> (Robineau-Desvoidy, 1830)
			<i>Calliphora pattoni</i> Aubertin, 1931
	Luciliinae	<i>Lucilia</i>	<i>Lucilia cuprina</i> (Wiedemann, 1830)
			<i>Lucilia sericata</i> (Meigen, 1826)
	Chrysomyinae	<i>Chrysomya</i>	<i>Chrysomya albiceps</i> (Robineau-Desvoidy, 1830)
			<i>Chrysomya bezziana</i> (Villeneuve, 1914)
			<i>Chrysomya megacephala</i> (Fabricius, 1794)
			<i>Chrysomya phaonis</i> (Seguy, 1928)
			<i>Chrysomya rufifacies</i> (Macquart, 1943)
	Polleniinae	<i>Pollenia</i>	<i>Pollenia rudis</i> (Robineau-Desvoidy, 1830)

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A preliminary checklist of the ants (Hymenoptera: Formicidae) of the Mt. Pantaron Range, Bukidnon Province, Mindanao Island, Philippines

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Abstract

The ants of a remote mountain in central Mindanao Island, Philippines are surveyed along a transect with a combination of collecting techniques. Nine species are new distributional records for the Philippines: *Aenictus wayani* Jaitrong and Yamane, 2011; *Aphaenogaster feae* Emery, 1889; *Euprenolepis procera* (Emery, 1900); *Myrmecina grandis* Okido, Ogata and Hosoishi, 2020; *Pheidole plinii* Forel, 1911; *Pheidole rabo* Forel, 1913; *Polyrhachis montana* Hung, 1970; *Ponera incerta* (Wheeler, 1933); and *Strumigenys doriae* Emery, 1887. Eighteen other species are new distributional records for the island of Mindanao.

Keywords: *Formicidae, Mindanao, new species record, Philippines.*

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Introduction

Biodiversity surveys are integral to the understanding of the biogeography of the ants of islands. The biogeography of the ants of the Philippine archipelago is far from clear, for at least three reasons.

First, the geology of the islands of the Philippines is complex. Most of the islands of the archipelago are oceanic, except for the Palawan Island Group (Lohman *et al.*, 2011). The two largest islands, Luzon and Mindanao, are hypothesized to be accretions of smaller proto islands (Corpuz, 1992; Hall, 2002; Heaney *et al.*, 2016). The complex geography of the islands may provide a wide range of microenvironments and opportunities for vicariance and dispersal of ants throughout the archipelago.

Second, the current knowledge of the diversity and distribution of ants in the Philippines is fragmentary. Many islands remain unexplored for ant diversity. Even the large islands of the central Philippines are scarcely explored. The current knowledge of the distribution of ant species by islands is also too coarse in resolution since the islands vary widely

in area and topology.

Third, only a scant handful of researchers are actively surveying the ant diversity of the Philippines. This, together with scarce funding, produces a patchy and incomplete view of the diversity and distribution of ants throughout the archipelago.

Despite limiting factors, even student thesis projects uncover species new to science, such as an undescribed species of *Meranoplus* F. Smith, 1853 from Dinagat Island, northeast of Mindanao Island (P. Buenavente, pers. comm.). Opportunities to survey the ants of the different islands of the Philippines are few and far between, especially during the pandemic, when travel is severely restricted. Hence, it is imperative to publish the results of any surveys immediately to provide building blocks of knowledge towards the understanding of the biogeography of the ants of the Philippines.

This current contribution, presents the preliminary results of an ant transect survey in an area that became an active conflict zone and is now off-limits to outsiders.

Materials and Methods

Study site

Mt. Bungkasan is a part of the Mt. Pantaron Range, the most extensive massif in central Mindanao Island (Amoroso *et al.*, 2020). The transect study was conducted near the boundary between the Provinces of Bukidnon and Agusan del Sur, in Sitio Mahayag, Saint Peter Village, Malaybalay City, Bukidnon Province, Mindanao Island (Fig. 1). The vegetation around the transect site is considered Tropical Upper Montane Rainforest and is

dominated by *Falcatifolium gruezoii* de Laub. (Podocarpaceae), *Agathis philippinensis* Warb. (Araucariaceae), and *Gymnostoma rumphianum* (Miq.) L.A.S. Johnson (Casuarinaceae) (N. Lagunday, pers. comm.). The transect line itself is in disturbed second-growth forest and crosses a clearing where a tree had just been felled and sawn into rough lumber, indicating that illegal logging is being committed in the area (Fig. 2). The coordinates at the camp site, which is near the transect line are: 8.263986111 N, 125.3303194 E, with an elevation of 1200 masl.



Figure 1: Approximate location of study site (red dot) near the boundary between the provinces of Bukidnon and Agusan del Sur on the Island of Mindanao. False color map indicates elevation from low elevation (green) to high elevation (red).

Collecting methods

Ants were collected using several methods. Ethanol (95% EtOH) was used to preserve all the specimens.

A modified and abbreviated ALL Protocol was applied (Agosti and Alonso, 2000), namely, Winkler extraction of ants from the leaf litter, pitfall trapping, twig and log breaking and opportunistic search through 10 sampling stations in a 100m horizontal transect. Opportunistic collecting was performed outside the transect, e.g., around the campsite.

Winkler extraction – leaf litter was gathered from a randomly selected 1m² area of forest floor, sifted the leaf litter in a sifting bag, and the siftate was placed in a Winkler bag for 48 hours.

Pitfall trapping – at a spot diametrically opposite the leaf litter collection site, a plastic cup (70mm diameter, 85mm depth) about half-filled with a weak soap solution (1-2 drops liquid dish detergent in 1 litre fresh water) was placed in the ground with the edge flush to the ground. The trap contents were retrieved after

24 hours to avoid maceration of the specimens. The soapy specimens were immediately rinsed in fresh water before they were transferred to 95% EtOH.

Twig and log breaking – rotten twigs and logs on the forest floor were broken open and those that contained ant nests were collected. The ants were collected either by hand or by Winkler extraction after the rotten woody material were first comminuted.

Opportunistic search – ants were collected within the transect during a 15-minute search period.

Identification

The ants were identified first to genus using published keys (General and Alpert, 2012; Schmidt and Shattuck, 2014). Then the following species-level keys for individual genera were used to determine species identifications: *Aenictus* (Jaitrong and Yamane, 2011, 2012); *Anochetus* (Brown, 1978); *Echinopla* (Xu and Zhou, 2015); *Euprenolepis* (LaPolla, 2009); *Gnamptogenys* (Lattke, 2004); *Myrmecina* (Okido *et al.*, 2020); *Myrmoteras* (Zettel and Sorger, 2011); *Pheidole* (Eguchi, 2001); *Polyrhachis* (*Polyrhachis*) (Kohout, 2014); *Ponera* (Taylor, 1967); *Recurvidris* (Bolton, 1992; Zettel, 2008); *Strumigenys* (Bolton, 2000); *Technomyrmex* (Bolton, 2007); *Tetramorium* (Bolton, 1976, 1977); and *Tetraponera* (Ward, 2001). Online resources were also consulted to confirm our determinations (AntWeb, 2021; AntWiki, 2021). All voucher specimens are deposited in the Entomological Collection of the University of the Philippines Los Baños Museum of Natural History (UPLB), the Natural History Museum of the Central Mindanao University (CMU), and the National Museum of the Philippines (NMP).

Imaging

Specimens were examined with a Leica S6D stereomicroscope. Images were created using a Leica MC120HD digital camera attached to the Leica S8APO stereomicroscope. These images were stacked using Combine ZM. The stacked images were edited with Adobe Photoshop CS5. The map was created in QGIS 3.14, using GIS data from PhilGIS 2021.



Figure 2: Area, where a tree was illegally felled and sawn into lumber, traversed by the transect line of this study.

Results

A total of 1,026 ants were collected, belonging to 75 species of 39 genera in seven subfamilies. Nine species are new distributional records for the Philippines: *Aenictus wayani* Jaitrong and Yamane, 2011; *Aphaenogaster feae* Emery, 1889; *Euprenolepis procera* (Emery, 1900); *Myrmecina grandis* Okido, Ogata and Hosoishi, 2020; *Pheidole plinii* Forel, 1911; *Pheidole rabo* Forel, 1913; *Polyrhachis montana* Hung, 1970; *Ponera incerta* (Wheeler, 1933); and *Strumigenys doriae* Emery, 1887.

The following 18 species are new distributional records for the Island of Mindanao: *Aenictus philippinensis* Chapman, 1963; *Anochetus graeffei* Mayr, 1870; *Calyptomyrmex beccarii* Emery, 1887; *Carebara maccus* (Wheeler, 1929); *Colobopsis vitrea* (F. Smith, 1860); *Cryptopone testacea* Emery, 1893; *Echinopla striata* F. Smith, 1857; *Ectomomyrmex leeuwenhoekii* (Forel, 1886);

Eurhopalothrix philippina Brown and Kempf, 1960; *Leptogenys peuqueti* (André, 1887); *Paraparatrechina iridescens* (Donisthorpe, 1942); *Pheidole fervens* (F. Smith, 1858); *Pheidole sayapensis* Eguchi, 2001; *Pheidole tjibodana* Forel, 1905; *Polyrhachis zopyra* F. Smith, 1861; *Ponera incerta* (Wheeler, 1933); *Strumigenys pedunculata* (Brown, 1953) and *Tetramorium insolens* (F. Smith, 1861).

Figs. 4 and 5 present the images of new Philippine records of species from this study. Fig. 6 presents the rank abundance plot of the collection.

Table 1 presents the list of species collected from the transect site and around the camp.

Behavioral Observations

Polyrhachis montana ants were observed to be foraging in the lumber slabs and sawdust (Fig. 2). They were then offered fish scraps, fish bones, and cooked rice as bait. The ants were strongly attracted to the bait and seemed to bite off small bits of fish bones (Fig. 3). One *P. montana* ant picked up a grain of cooked rice and walked uphill, up a tree, along a vine into the canopy. The ant was about 70 meters from the bait when it was lost to view. There was no evidence of group transport of larger bait items.

On another log, *Pheidole singaporensis* ants dominated some chicken bones as bait. The next day, the same bones, were being processed by a smaller (indeterminate) species of *Pheidole*.

Table 1. Preliminary checklist of the ants of Mt. Bungkasan, St. Peter Village, Malaybalay City, Bukidnon Province, Mindanao Island, Philippines

No.	Species	Transect	Camp
	Subfamily Dolichoderinae		
1	<i>Dolichoderus thoracicus</i>	B	
2	<i>Technomyrmex sundaicus</i>	W	
	Subfamily Dorylinae		
1	<i>Aenictus philippinensis</i> *	O	
2	<i>Aenictus wayani</i>	O	
3	<i>Chrysapace jacobsoni</i>	W	
	Subfamily Ectatomminae		
1	<i>Gnamptogenys binghamii</i>	O	
2	<i>Gnamptogenys</i> sp. undet1	W	
	Subfamily Formicinae		
1	<i>Camponotus</i> sp. undet1	T	
2	<i>Camponotus</i> sp. undet2	B	
3	<i>Camponotus</i> sp. undet3		O
4	<i>Colobopsis corallina</i>	B, O	O
5	<i>Colobopsis vitrea</i> *	B, T	
6	<i>Echinopla striata</i> *		O
7	<i>Euprenolepis procera</i>	W, T, P	
8	<i>Lepisiota chapmani</i>	B	
9	<i>Myrmoteras insulcatum</i>	W	
10	<i>Nylanderia</i> sp. undet1	W	
11	<i>Paraparatrechina iridescens</i> *	B, T	

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12	<i>Polyrhachis armata</i>	B	O
13	<i>Polyrhachis montana</i>	O	
14	<i>Polyrhachis zopyra</i> *		O
15	<i>Pseudolasius</i> sp. undet1	W, T	
	Subfamily Myrmicinae		
1	<i>Acanthomyrmex mindanao</i>	W	
2	<i>Aphaenogaster feae</i>	W, T	
3	<i>Calyptomyrmex beccarii</i> *	W	
4	<i>Carebara diversa</i>		O
5	<i>Carebara maccus</i> *	W, T, P	
6	<i>Carebara</i> sp. undet1	W	
7	<i>Crematogaster brunnea ruginota</i>		O
8	<i>Crematogaster</i> sp. undet1	B	
9	<i>Crematogaster</i> sp. undet2	B	
10	<i>Eurhopalothrix philippina</i> *	W	
11	<i>Myrmecina grandis</i>	W	
12	<i>Myrmicaria aphidicola</i>	P, O	O
13	<i>Pheidole aglae</i>	W, T, P	
14	<i>Pheidole fervens</i> *	B	
15	<i>Pheidole jacobsoni</i>	T	
16	<i>Pheidole kikutai</i>	O	
17	<i>Pheidole parva</i>	O	O
18	<i>Pheidole plinii</i>	O	
19	<i>Pheidole quadricuspis</i>	W	
20	<i>Pheidole rabo</i>	W	
21	<i>Pheidole retivertex</i>	W	
22	<i>Pheidole sayapensis</i> *	W	
23	<i>Pheidole singaporensis</i>	W, P, O	
24	<i>Pheidole</i> sp. undet1	W	
25	<i>Pheidole tjibodana</i> *	W	
26	<i>Recurvidris</i> sp. undet1	W	
27	<i>Strumigenys arrogancia</i>	W	
28	<i>Strumigenys doriae</i>	W	
29	<i>Strumigenys koningsbergeri</i>	W	
30	<i>Strumigenys pedunculata</i> *	W, T	
31	<i>Strumigenys</i> sp. undet1	W	
32	<i>Strumigenys</i> sp. undet2	W	
33	<i>Tetramorium insolens</i> *	W, B	
34	<i>Tetramorium katypum</i>	W, B	

35	<i>Tetramorium pacificum</i>	B, O	
36	<i>Tetramorium wroughtonii</i>	W	O
37	<i>Vollenhovia ambitiosa</i>	W, P, O	
38	<i>Vollenhovia</i> sp. undet1	W, T	
39	<i>Vollenhovia</i> sp. undet2		O
40	<i>Vombisidris</i> sp. undet1	B	
	Subfamily Ponerinae		
1	<i>Anochetus graeffei</i> *	W	
2	<i>Brachyponera obscurans</i>	W, T, P	
3	<i>Brachyponera</i> sp. undet1	W	
4	<i>Cryptopone testacea</i> *	W, P	
5	<i>Ectomomyrmex leeuwenhoekii</i> *	W	
6	<i>Hypoconera</i> sp. undet1	T	
7	<i>Hypoconera</i> sp. undet2	W, O	
8	<i>Leptogenys peuqueti</i> *	P	
9	<i>Leptogenys</i> sp. undet1	W	
10	<i>Odontoponera denticulata</i>		O
11	<i>Ponera incerta</i>	W	
12	<i>Pseudoponera stigma</i>	W	
	Subfamily Pseudomyrmecinae		
1	<i>Tetraponera allaborans</i>		O

Ants were collected at either the transect site or the camp and environs. Collection techniques used were B = Beating of low vegetation; O = Opportunistic collecting; P = Pitfall trapping; T = Twig or wood breaking; and W = leaf litter sifting and Winkler extraction. Species names in boldface are new distributional records for the Philippines. Species names with * are new distributional records for the Island of Mindanao.

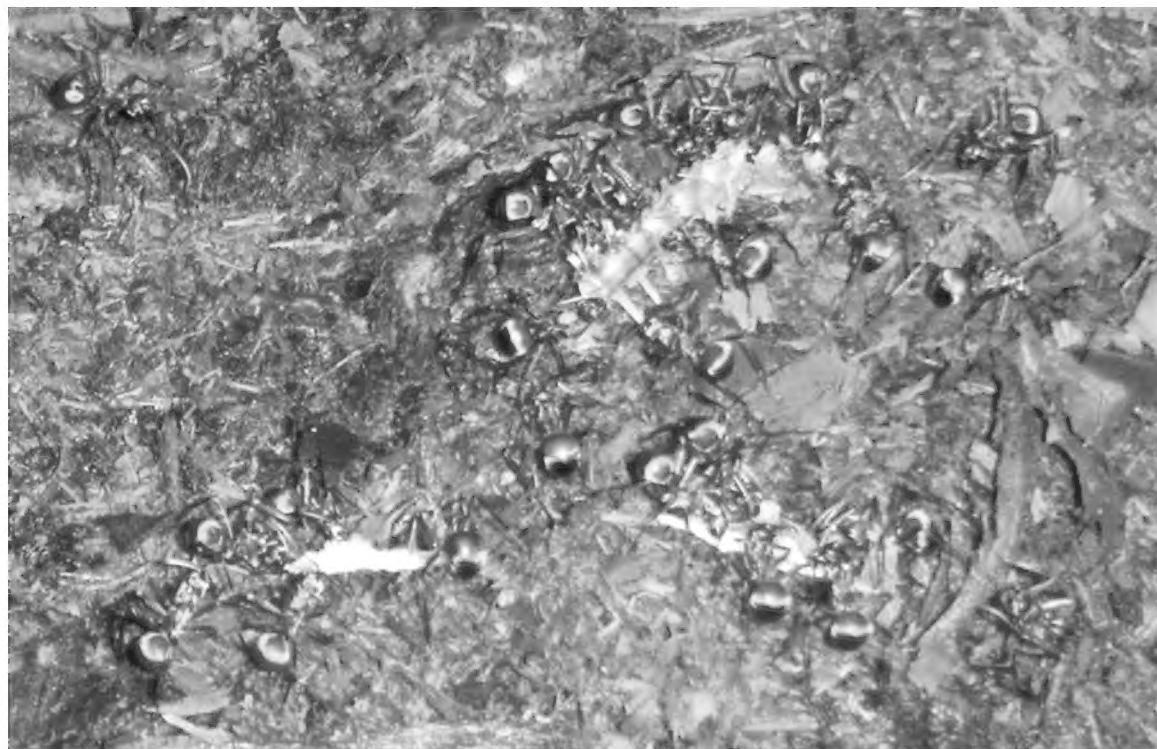


Figure 3: *Polyrhachis montana* ants at baits of cooked rice and fish bones

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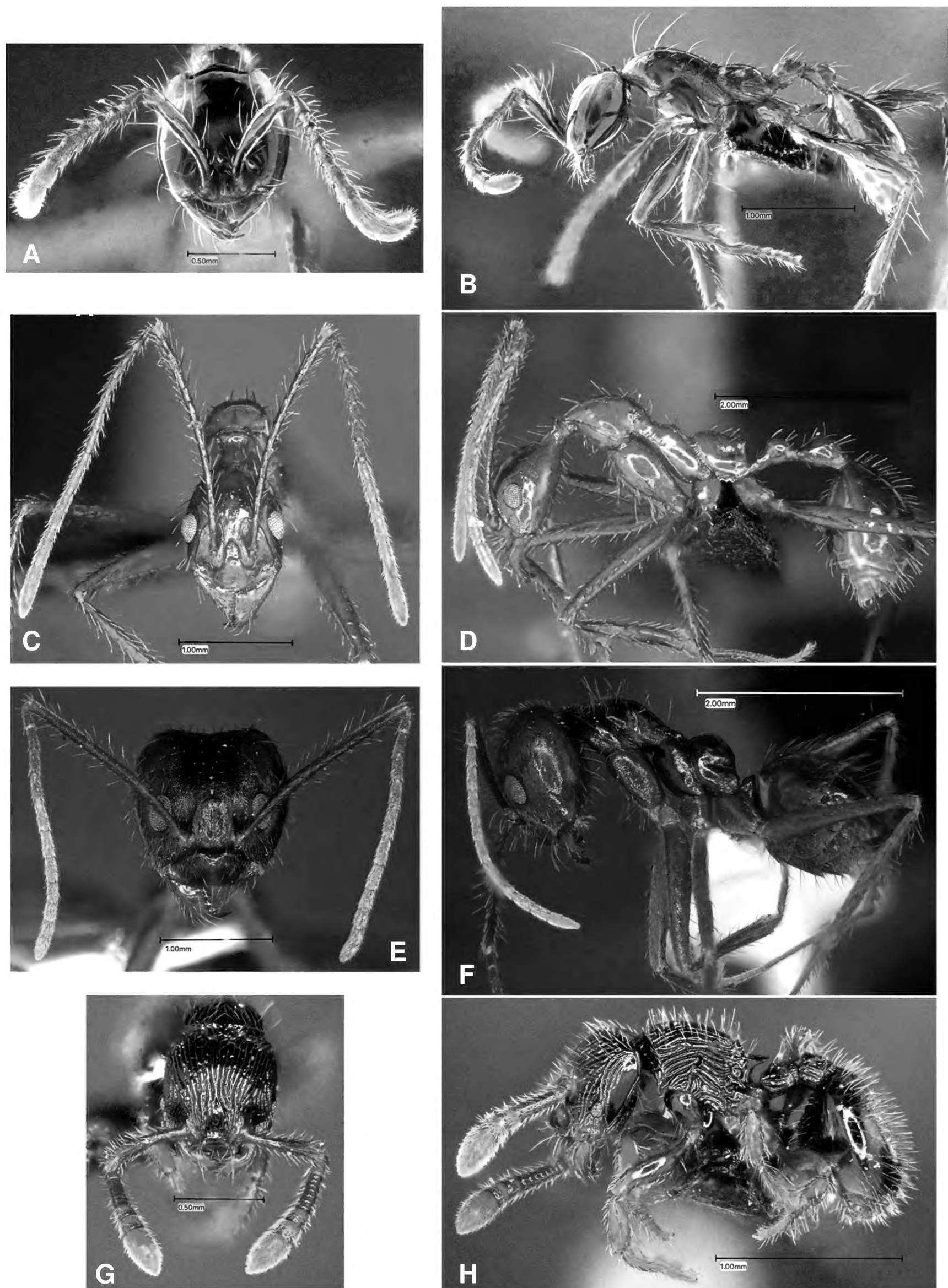


Figure 4: Some new Philippine records of species from Mt. Bungkasan, Bukidnon Province, Mindanao Island, Philippines, full-face view and lateral habitus. **A-B:** *Aenictus wayani*; **C-D:** *Aphaenogaster feae*; **E-F:** *Euprenolepis procera*; **G-H:** *Myrmecina grandis*.

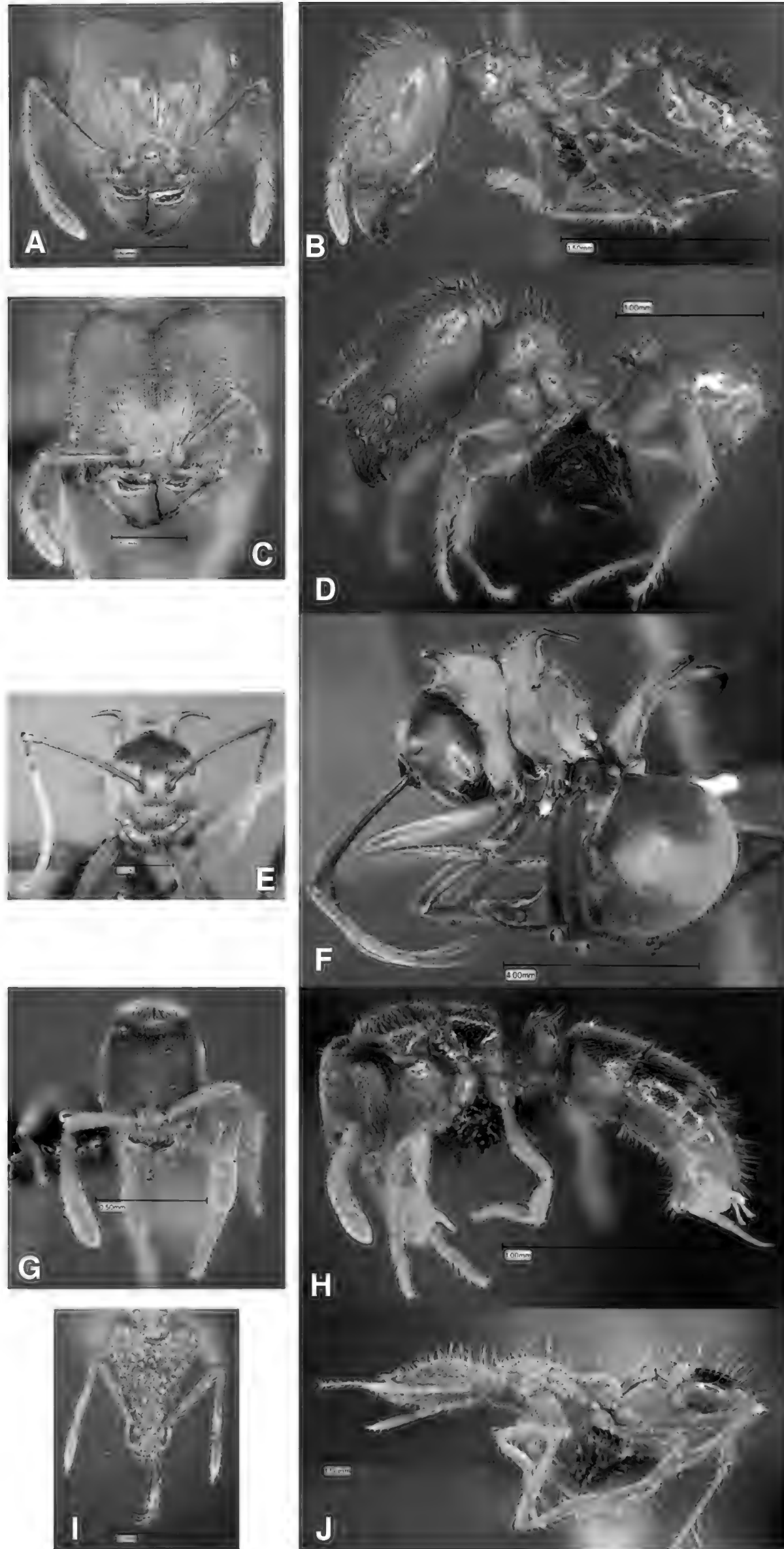


Figure 5: Some new Philippine records of species from Mt. Bungkasan, Bukidnon Province, Mindanao Island, Philippines, full-face view and lateral habitus. **A-B:** *Pheidole plinii*; **C-D:** *Pheidole rabo*; **E-F:** *Polyrhachis montana*; **G-H:** *Ponera incerta*; **I-J:** *Strumigenys doriae*.

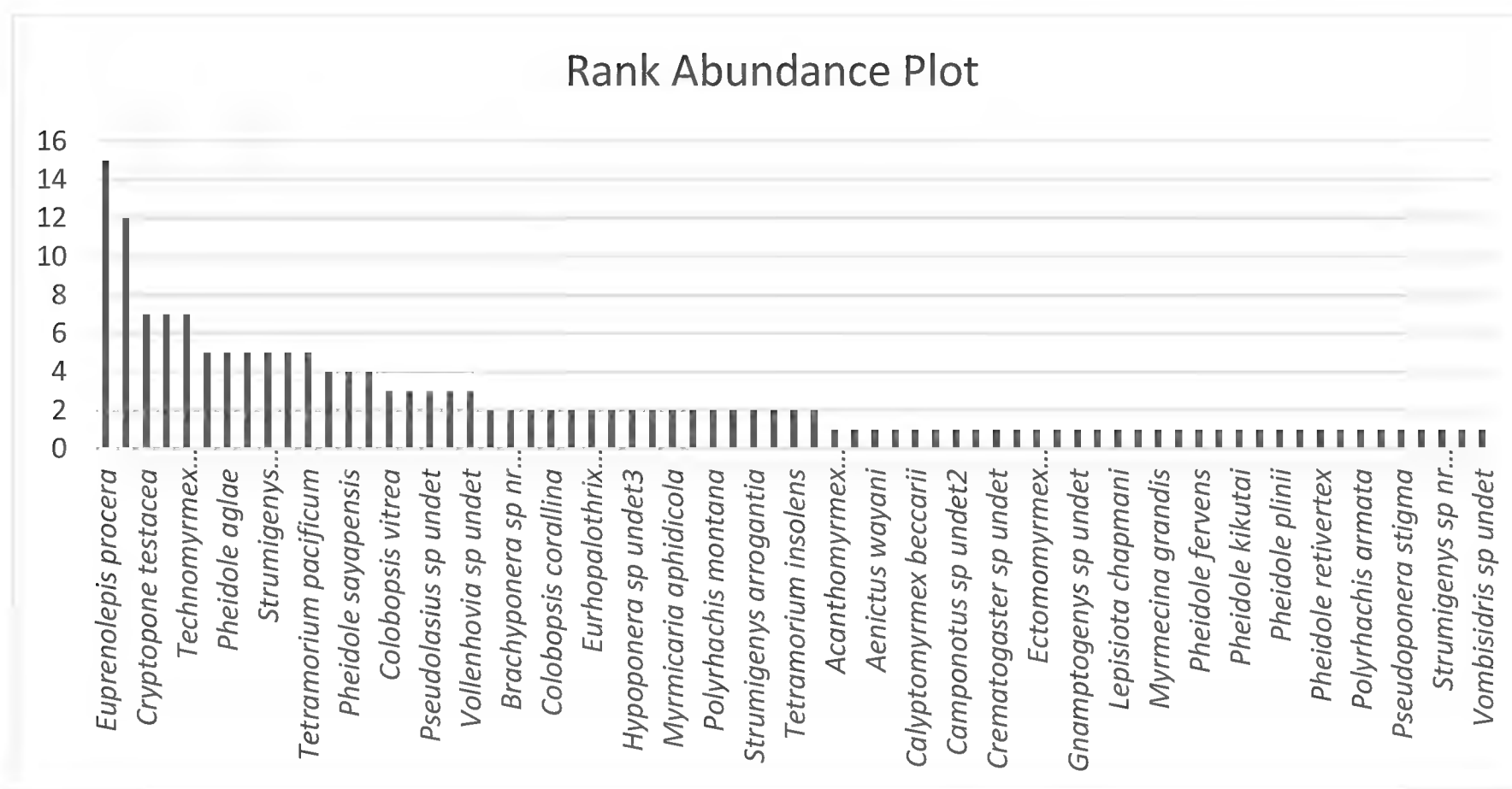


Figure 6: Rank abundance plot of ant collection. The horizontal axis is the partial list of species arranged from the highest number of occurrences in the pooled dataset. The vertical axis is the number of occurrences, or number of times the species was collected in the transect, combining all the collecting techniques.

Discussion

This current contribution provides another small look at the diversity of ants on Mindanao Island, in particular, and the Philippines, in general. The number of new country records and new island records of species is remarkable considering that only one transect was conducted. Unfortunately, the study site is now closed to researchers because it has become an active conflict area. The results suggest that Mindanao is a rich hunting ground for ant diversity studies.

Biodiversity surveys in unexplored regions, such as the mountainous central Mindanao, almost always discover new species records and potentially new species. This current study is no exception, having recorded nine new Philippine distributional records of species and 18 new distributional records for the island of Mindanao.

This study is another small building block for understanding the biogeography of the ants of the Philippines.

Conclusion

The ant diversity of Mindanao Island.

despite being the second largest island of the Philippines, is poorly studied. The researcher must overcome many challenges to conduct field work in Mindanao, including a dangerous insurrection. Returning to the study site to conduct further field work is currently impossible. It is uncertain when it becomes safe again to visit the area.

This study is a fortunate output of a collaborative biodiversity survey project among three research institutions, namely UPLB, CMU, and NMP, in the Philippines. Nevertheless, the project itself proceeded haltingly because of the pandemic. And more surveys of Mindanao Island are needed to further elucidate the fine-scale distribution of ants on the island.

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First record of two Doryline ant genera *Cerapachys* Smith, 1857 and *Parasyscia* Emery, 1882 (Hymenoptera: Formicidae) from Nepal

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Abstract

The presence of two ant genera, *Cerapachys* Smith, 1857 and *Parasyscia* Emery, 1882 is reported for the first time in Nepal. We collected two workers of *Parasyscia wighti* (Bharti & Akbar, 2013) from Ranibari Community Forest and one worker of *Cerapachys sulcinodis* Emery, 1889 from Nagarjun forest using pitfall traps. With this discovery, the total number of Doryline ant genera of Nepal has reached to five. Synoptic account of these two genera, worker description of the species and identification keys to Nepalese genera of the subfamily Dorylinae are provided. Images of all known species representing full-face, dorsal and profile views are provided.

Keywords: *Army ants, Dorylinae, Nepalese ants, new records, petiole.*

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Introduction

Ants (Hymenoptera: Formicidae) are one of the world's most successful insect groups, dominating in population, geography and ecology (Hölldobler & Wilson, 1990). They can be found in almost all terrestrial environments, with the greatest diversity in tropical areas (Guénard, 2013). Ants are frequently regarded as superorganisms due to their dominance in the environment (Hölldobler & Wilson, 2009). Ants have been classified into 17 subfamilies, 338 genera, and 13,981 species (Bolton, 2021), with thousands of undescribed taxa. Nepal has eight subfamilies, 48 genera, and 133 species formally recorded (Subedi *et al.*, 2020; 2021), but given the country's unique geography and ecological diversity with large unexplored areas, this figure is far from a complete list and many ant species have yet to be identified and described.

Dorylinae includes a monophyletic group of predatory ants found in tropical, subtropical, and warm temperate regions of the world (Borowiec, 2016) with 27 valid genera

and 735 species (Bolton, 2021). Within the subfamily, these ants are highly diverse in both habits, morphology, nesting sites (subterranean or arboreal nests), and colony size, with a few dozen to millions of workers in a colony (Borowiec, 2016). Workers are either blind or have small to well-developed compound eyes, have short and long slender appendages, and coarse to shiny cuticle. Army ants are a fascinating group of organisms characterized by morphological and behavioral adaptations such as obligate collective foraging, nomadism and highly specialized queens (Brady, 2003; Brady *et al.*, 2014). Borowiec (2019) revealed through phylogenetic studies that the army ants syndrome exemplifies both long-term evolutionary stasis and a remarkable case of convergent evolution. Dorylinae includes a variety of ants in addition to true army ants, which were previously kept in the subfamilies Cerapachyinae and Leptanilloidinae before the study of Brady *et al.* (2014). Borowiec (2016)

revised the generic level classification of Doryline ants, creating a total of 28 genera, up from 19 in Brady *et al.* (2014).

Doryline ants are currently represented in Nepal by three genera: *Aenictus*, *Dorylus*, and *Ooceraea*, and four species: *A. sagei*, *D. labiatus*, *D. orientalis*, and *O. biroi* (Subedi *et al.*, 2020). The current study reports two rare ants, *Cerapachys sulcinodis* and *Parasyscia wighti*, bringing the total number of Doryline ant genera reported from Nepal to five. This paper aims to provide a synoptic account of two Doryline ant genera recorded for the first time from Nepal, *Cerapachys* Smith, 1857 and *Parasyscia* Emery, 1882. It also presents a worker description of the reported species and provides a key to the Nepalese Doryline ant genera. Photo images (full-face, dorsal, and profile views) are provided to validate these new faunal records from Nepal and to aid in their identification.

Materials and Methods

The specimens for this study were collected from Nagarjun forest, Shivapuri-Nagarjun National Park and Ranibari Community Forest (RCF) in 2019. Out of over 4000 ant specimens collected by using 160 pitfall traps, 160 bait traps at four different sites, and hand collecting along foot trails from Fulbari gate to Jamacho of Nagarjun forest in autumn and spring, and 40 pitfall traps, 40 bait traps, and hand collecting at RCF, only one specimen of *Cerapachys* and two specimens of *Parasyscia* were obtained in pitfall traps. The collected specimens were preserved in 90% alcohol before being point-mounted. A stereo zoom microscope (Coslab MSZ-115) was used to examine the morphology of the point-mounted specimens. Images were captured using a digital camera (Samsung SM-M625F), under the same microscope. The specimens are deposited at Central Department Zoology Museum of Tribhuvan University (CDZMTU). Identifications are based on available keys (Bharti & Akbar, 2013), the original description, and a comparison with images available on AntWiki (<https://www.antwiki.org>). A synoptic generic account of both genera is provided, including type species, type locality, junior synonyms, diagnostic features, distribution, and

biology, followed by worker description of Nepalese species.

Results and Discussion

Out of 50 ant genera known from Nepal, a few individuals of the genus *Cerapachys* (one individual) and *Parasyscia* (two individuals) were collected in pitfall traps from Nagarjun and RCD respectively. The type information, distribution, and diagnostic features of newly recorded genera and species from Nepal are provided below. The identification key to Nepalese ant genera of the subfamily Dorylinae is also presented.

Cerapachys Smith, 1857

= *Ceratopachys* Schulz, 1906

= *Cysias* Emery, 1902

= *Neophyracaces* Clark, 1941

= *Phyracaces* Emery, 1902

Type species: *Cerapachys antennatus* Smith, 1857, by subsequent designation of Bingham, 1903: 28.

Type locality: Borneo (East Malaysia: Sarawak)

Cerapachys is a small genus with five known species (Bolton, 2021). Its range extends from northwest India and Tibet to southern China, Java, Borneo, and the Philippines (Borowiec, 2016). The genus has been placed in different subfamilies: Myrmicinae (Smith, 1857), Ponerinae (Dalla Torre, 1893), and Cerapachyinae (Wheeler, 1902). Based upon phylogenetic studies, Brady *et al.* (2014) confirmed it as a Doryline genus. Borowiec (2016) also placed it in Dorylinae.

These non-army ant dorylines can be diagnosed by the presence of spiracle below the propodeum's mid-height, well-developed pygidium having modified setae, a prominent pronotomesopleural suture, pronotal collar with a well-developed carina, mid and hind tibia each with a single pectinate spur and a helcium located supra axially in the upper half of the third abdominal segment (Borowiec, 2016).

Cerapachys species build their nests in rotting logs and wood fragments, under stones, leaf litter, and soil. Some species appear to be semi-nomadic, with much larger colonies than

cryptic congeners, whose colonies may not exceed one hundred (Eguchi *et al.*, 2014). The common methods for collecting these ants include breaking open twigs, sifting leaf litter, and looking for columns of raiding workers.

***Cerapachys sulcinodis* Emery, 1889**

= *Cerapachys butteli* Forel, 1913

= *Cerapachys risii* Forel, 1892

(Figs. 1, 2, 3)

Type locality: Myanmar (Tenasserim, Mt. Mooleyit).

Distribution: Nepal (New record), Himalaya, SE Asia, Philippines and Sumatra, Borneo (Brown, 1975), Myanmar, Malaya peninsula (Bingham, 1903), Vietnam (Radchenko, 1993), Thailand (Jaitrong & Nabhitabhata, 2005), China (Guenard & Dunn, 2012), India (Bharti & Akbar, 2013), Laos (Jaitrong *et al.*, 2016).

Materials examined: 1 worker, Nagarjun forest, Shivapuri-Nagarjun National Park, 27.74871N, 85.27361E, 1912 m, pitfall collection, 13-15.iv.2019, IP Subedi leg., CDZMTU.

Worker description:

Head distinctly longer than broad; posterior cephalic margin roughly straight, eyes moderately large, mandibles triangular, masticatory margin with small denticles, 12-segmented clavate antennae.

Mesosoma stout and compact, roughly rectangular, slightly rounded sides with no distinct margin, propodeal declivity concave medially, propodeal spiracle elongate. Petiole longer than broad, petiolar dorsum rounding into sides, dorsal surface of petiolar node with longitudinal grooves and smooth median area, subpetiolar processes peg like, postpetiole trapezoidal and broader than long, elongated gaster, sting exerted.

Sculpture and pilosity: Body shiny with widely scattered, indistinct punctures throughout the body except gaster. Body moderately pilose with long erect or sub-erect hairs typically prominent in head, postpetiole and gaster.

Body coloration: The workers are small, shiny black in color with brown legs and antennae.

Comments: It is very rare in Nepal, having been discovered only once in a pitfall trap in Nagarjun forest during an extensive survey using multiple sampling methods.



Figures 1-3: *Cerapachys sulcinodis*: 1. Profile view; 2. Head in full-face view; 3. Dorsal view.

***Parasyscia* Emery, 1882**

Type species: *Parasyscia piochardi*, by monotypy.

Type locality: Syria

Parasyscia is represented by 51 species (Bolton, 2021) that are found throughout the old world's warm temperate and tropical regions (Borowiec, 2016) and a few species in the subtropics (Fisher & Bolton, 2016). The genus *Parasyscia* has been classified as Ponerinae (Dalla Torre, 1893) and as Dorylinae (Emery, 1895). It was described as genus by Emery (1882) and classified as a subgenus of *Cerapachys* (Forel, 1892), and as a junior synonym of *Cerapachys* (Kempf, 1972). Borowiec (2016) revived it as a valid genus of the subfamily Dorylinae after a thorough revision of the subfamily's generic-level classification.

Borowiec (2016) recognized the following characteristics to diagnose *Parasyscia* workers: Propodeal spiracle positioned low on the sclerite, propodeal lobes present. Constriction between III and IV abdominal segments but no constrictions between IV, V, and VI segments. Petiole dorsolaterally not marginate. Pronotomesopleural suture fused. Helcium axial. Middle tibiae with a single pectinate spur, pretarsal claws unarmed, and abdominal segment III anterodorsally often marginate.

The genus includes small, cryptic ants found in rotting logs, under stones (Brown, 1975), arboreal nests (Sarnat & Economo, 2012), and urban environments (Borowiec, 2016). Their colonies appear to have a small number of individuals (Borowiec, 2016).

***Parasyscia wighti* (Bharti & Akbar, 2013)**

(Figs. 4, 5, 6)

Type locality: India (Silent Valley National Park, Kerala).

Distribution: Nepal (New record), India (Bharti & Akbar, 2013).

Materials examined: 2 workers, RCF, Kathmandu, 27.729444N, 85.320555E, 1310 m, pitfall collection, 13-15.x.2019, IP Subedi leg., CDZMTU.

Worker description

Head rectangular, longer than broad; slightly rounded posterior lateral corners, posterior cephalic margin transverse, prominent parafrontal ridges, eyes reduced, mandibles subtriangular, masticatory margin with no denticles, 12-segmented clavate antenna with short scape.

Mesosoma compact, rectangular in dorsal view, slightly convex dorsal surface, slightly rounded sides with no distinct margin, propodeal declivity smooth and marginate. Petiole longer than broad, subpetiolar processes with hook like ventral margin, sub trapezoidal postpetiole slightly wider behind, elongated gaster.

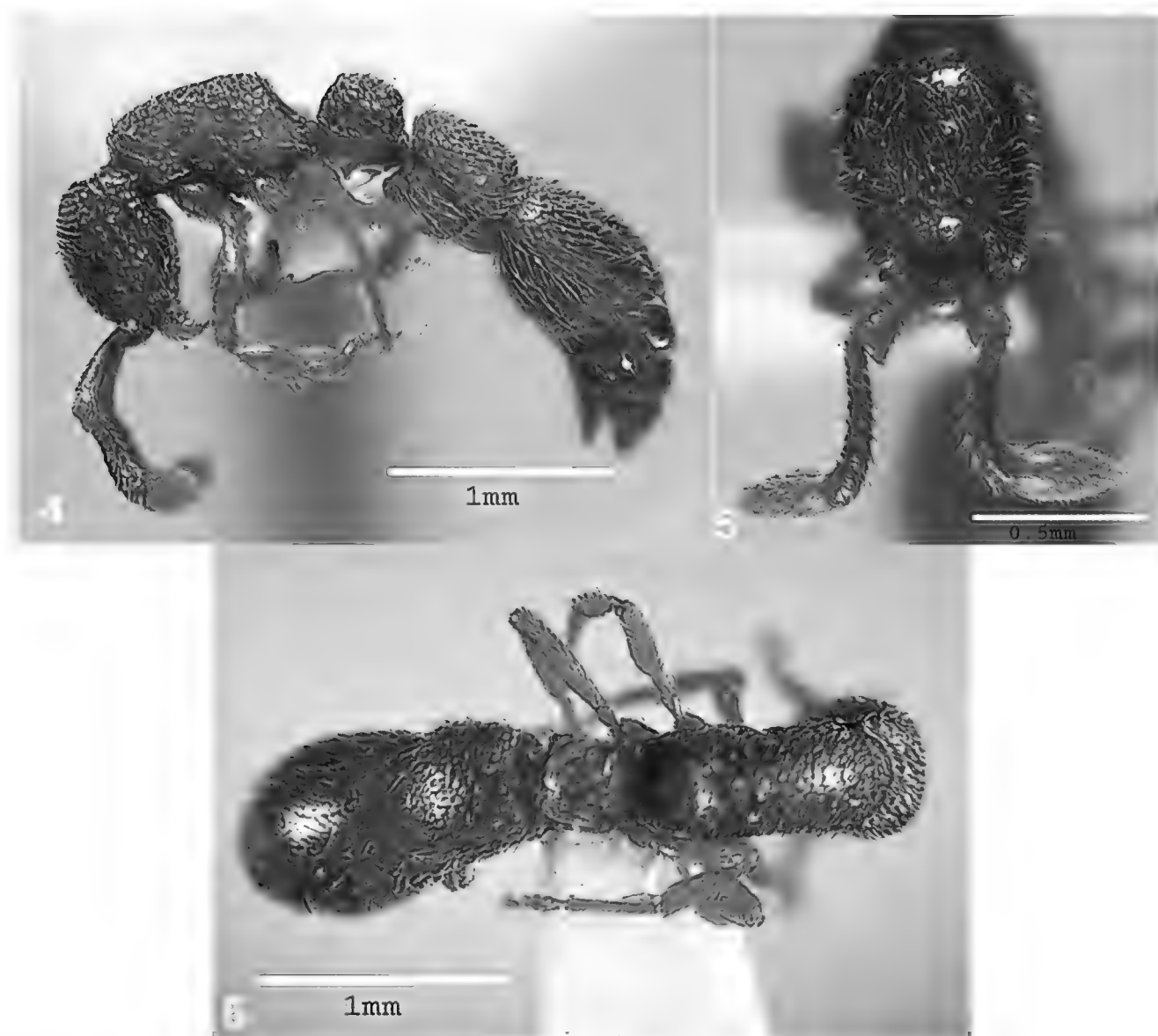
Sculpture and pilosity: Large and crowded punctures on the head dorsum, with diameters equal to greater than the average distance between them. Mesosoma, petiole, and postpetiole with similar sculpture, smaller punctures on gaster. Body moderately pilose with decumbent or subdecumbent hairs typically prominent in postpetiole and gaster. Standing hairs on the apical funicular segments and legs.

Body coloration: The body is mostly dark reddish brown in color, with slightly lighter mandibles, antennae, and legs.

Comments: It is extremely rare in Nepal, having been discovered only once in a pitfall trap during an extensive survey using multiple sampling methods and it is most likely cryptic in nature.

Key to Nepalese Dorylinae ant genera based on worker caste

The following key to Nepalese ant Doryline ant genera is mainly based upon Borowiec (2016). The numbers in parentheses refer to the previous couplet in the sequence. The genus *Syscia* has not yet been documented in Nepal, but it is included in the key for comparison purposes.



Figures 4-6: *Parasyscia wighti*: **4.** Profile view; **5.** Head in full-face; **6.** Dorsal view.

1. Pygidium, not armed with numerous modified setae, at most with only one or two pairs of thick setae or cuticular projections. Propodeal lobes short or absent. Propodeal spiracle positioned high on the propodeum 2
- Pygidium armed with numerous specialized, peg-like or spiniform setae much thicker than surrounding fine hairs; setae more than four in number, often more numerous. If pygidium is small or with few specialized setae, then propodeal lobes are conspicuous. Propodeal spiracle positioned low or mid-height on the propodeum.....3
2. (1) Antennae with 8–10 segments. Binodal waist. Pygidium small, reduced to narrow strip, without impressed medial field and simple, not armed with cuticular spines or modified setae. *Aenictus*
- Antennae with 8–12 segments. Uninodal waist. Pygidium large and impressed at apex, armed with one or two cuticular teeth or spines on each side.....*Dorylus*
3. (1) The pronotomesopleural suture is either fully or partly fused in lateral view, and there is never a curving incision in the cuticular surface that approaches the dorsolateral borders of the pronotum..... *Parasyscia*
- Pronotomesopleural suture appears as a deep incision in the cuticle in lateral view, typically curled below the dorsolateral borders of the mesosoma.....4
4. (3) Helcium circumference is enormous in comparison to abdominal segment II (petiole) and is located above the segment's midheight, resulting in a low, undifferentiated rear face of the petiole and a low anterior face of abdominal segment III.....*Cerapachys*
- Helcium circumference is modest in comparison to abdominal segment II (petiole), which is positioned at around the midpoint of the segment, resulting in a distinct rear face to abdominal segment II and a prominent anterior face to abdominal segment III.....5
5. (4) Abdominal segment III is relatively narrow in dorsal view and similar in size to the petiole. In lateral view, abdominal tergite IV not folding over sternite and the anterior portion of the sternite visible. Hind

basitarsi not dilating distally, circular in cross-section.....*Ooceraea*

- Abdominal segment III is relatively wide in dorsal view and larger than the petiole. In lateral view, abdominal tergite IV folding over sternite and the anterior portion of sternite at least partly obscured. Hind basitarsi swollen at about two thirds of their length, oval in cross-section.....*Syscia*

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First Record of *Italochrysa japonica* (McLachlan, 1875) (Neuroptera: Chrysopidae) from India

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Abstract

The species, *Italochrysa japonica* (McLachlan, 1875) belonging to the Chrysopidae family is recorded for the first time from India.

Keywords: *Italochrysa*, *Chrysopidae*, *India*.

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Introduction

The order Neuroptera, commonly known as ‘lacewings’ is a relatively small group of insects, with 6000 species known globally (Oswald, 2021). Among this, about 327 species which belongs to 115 genera and 12 families are known from India (Singh *et al.*, 2020).

Chrysopidae (also known as green lacewing), globally comprises 1200 species belonging to 80 genera of which 66 species under 21 genera are known from India (Brooks and Barnard, 1990; Chandra and Sharma, 2009). The family includes three extant subfamilies; Chrysopinae, Nothochrysinae and Apochrysinae which are also recorded from India (Ghosh, 2000; Chandra and Sharma, 2009). All the species in their larval stage and adults of few genera are predators of aphids, coccids, and other soft-bodied insects. Thus, they are successfully used as biological control agents of agricultural pests (New, 1975). Even though their roles as biological control agents are well documented, their taxonomic studies are insufficient and there is a high possibility of description and redescription of new taxa from the country.

Chrysopinae is the largest of the three subfamilies of Chrysopidae in which the Belonopterygini is the most primitive tribe. It retains many plesiomorphic characters not found in other groups (Brooks and Barnard, 1990). Although 14 genera are assigned to Belonopterygini in the world, only 4 genera and 11 species are reported from India (Brooks

and Barnard, 1990; Chandra and Sharma, 2009). *Italochrysa* Principi, 1946 belongs to Belonopterygini which comprises nearly 90 species in the world. Larvae of *Italochrysa* live parasitically in ant-colonies. They feed on the larvae and pupae of the ants while the adults will not feed on any insects (Principi, 1946). In India, only 7 species of *Italochrysa* (*I. aequalis aequalis* (Walker, 1853) from Karnataka, *I. carletoni* (Banks, 1939) from Himachal Pradesh and Sikkim, *I. flavobrunnea* Ghosh, 1981 from Madhya Pradesh and Tamil Nadu, *I. henryi* (Kimmins, 1938) from Kerala, *I. lefroyi* (Needham, 1909) from Assam, Bihar, Meghalaya and Punjab, *I. robusta* (Needham, 1909) from Assam, *I. talaverae* (Navás, 1928) from Sikkim) are reported (Chandra and Sharma, 2009; Oswald, 2021). Incidentally, scanning of the literature revealed that *I. japonica* (McLachlan, 1875) was never recorded from India. Thus in this study, we report *I. japonica* for the first time from India.

Materials and Methods

The specimens were collected using a sweep net. The collected specimens were killed using a killing jar with 2 to 3 drops of Ethyl acetate. Later, specimens were dried and held on entomological pins with proper labelling. The specimens were examined under Leica M205 Stereomicroscope and identified using taxonomic keys of Kim and Cho, 2015. The digital images of the specimens were taken with a Nikon Coolpix P900 with Raynox

250 lens. The specimens were deposited in the Insect Collections of Shadpada Entomology Research Lab (SERL), Kerala, India.

Results

Diagnosis

Italochrysa Principi, 1946

Principi, *Boll. Ist. Ent. Univ.* 15: 86, 1946.

Italochrysa can easily be identified from other Beleopterigini by the presence of a long quadrangular intramedian cell in the forewing (Fig. 2).

Italochrysa japonica (McLachlan, 1875)

McLachlan, *Trans. Royal. Ent. Soc. Lond.* 23: 182, 1875.

Italochrysa japonica is a fascinating medium-sized species (8-12 mm) characterised by red horizontal lines in pronotum and abdomen white with clamp shaped black markings at each segment (Fig. 1).

Description

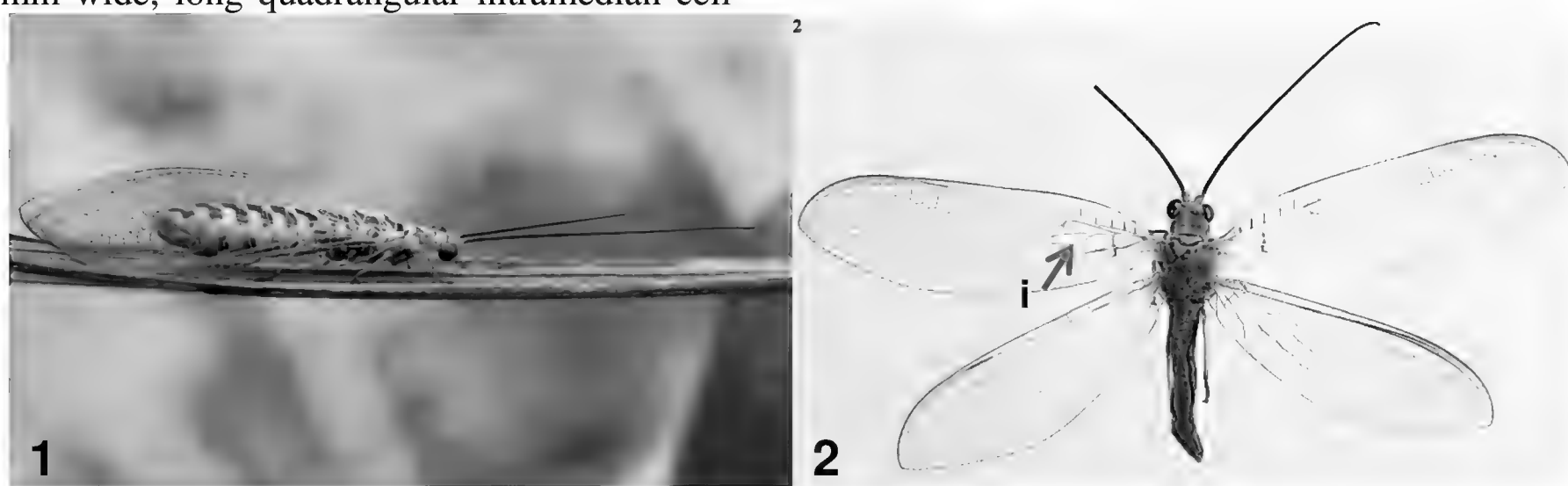
Female: Head and two basal segments of antennae yellow and rest of antennal segments deep black. Length 12 mm from head to abdomen and 2 mm wide. Pronotum white with red horizontal lines. Mesonotum and metanotum white with large black spots on sides. Wings narrow elongate and veins yellowish-white. Forewing 17 mm long and 5 mm wide, long quadrangular intramedian cell

present. Pterostigma long and narrow with light brown colour. Hindwing 15 mm long and 4 mm wide. Abdomen white with clamp shaped black markings at each segment. Female easily distinguished from males by their broad abdominal tip and bilobed subgenitales and a pillbox-shaped spermatheca with long coiled duct.

Remarks: The live specimen has a bright black and white colour on the dorsal side of thorax and abdomen but when the specimen gets dried the colour of the specimen completely changes to brownish-black (Figs. 1 and 2).

Materials examined: 1♀, India: Kerala, Thrissur district, Palakkal, 10°25'55.06"N, 76°20'14.72"E, 20-VIII-2020, Coll. Suryanarayanan T.B., SERLNR106; 1♀, Kerala, Kollam district, Rosemala, 8°54'53.58"N, 77°10'11.91"E, 28-III-2021, Coll. Suryanarayanan T.B., SERLNR107; 2♀, Kerala, Thrissur district, Mannuthy, 10°32'06.20"N, 76°15'43.81"E, 02-VI-2021, Coll. Suryanarayanan T.B., SERLNR108, SERLNR109.

Distribution: China, Japan, Korea and India (McLachlan, 1875; Kim and Cho, 2015; Oswald, 2021).



Figures 1-2: *Italochrysa japonica* (McLachlan, 1875): 1. Live adult female; 2. Habitus (i. quadrangular intramedian cell)

Discussion

Chrysopidae is a beneficial candidate of Order Neuroptera with their carnivorous habit (feeding on pests in larval and adult stages). Even though they are widely used as biological pest control agents, their taxonomy is least studied in India. Non-taxonomists get easily confused during the identification of the

specimens belonging to this group mainly because of the variation in specimen colour in live and dried condition and many species are superficially similar (Brooks, 1983). Literature showed a definite lack of record of *Italochrysa japonica* from Indian region. The present article reports *Italochrysa japonica* from India for the first time. It also aims to give an eye-

opener for the researchers on future exploration studies on the Chrysopidae family and their application as biological pest control agents.

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Mixed colonies of *Lasius umbratus* and *Lasius fuliginosus* (Hymenoptera, Formicidae): when superparasitism may potentially develop into coexistence: a case study in Ukraine and Moldova

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Abstract

In 2015, in the territory of Kyiv (Ukraine) and in 2012-2017 in Transnistria (Moldova), two mixed colonies consisting of two species of ants – *Lasius fuliginosus* and *L. umbratus* were observed. Another 2 mixed colonies were discovered in 2021 in the territory of the city of Rivne (Ukraine). The aim of the study was to describe the observed mixed colonies, analyze the interaction of workers of two species on the trails, and by mathematical modelling to determine the probability of preserving queens of both species as part of a mixed colony. On the trails of the colonies from Kyiv and Rivne, the number of workers of *L. fuliginosus* is slightly higher than that of *L. umbratus*, so is the number of *L. fuliginosus* workers in the colony. Workers of both species were making contact both within the same species and interspecific contacts, but trophobiosis with aphids has been recorded only by *L. fuliginosus*. The most likely of the 4 options considered was the survival of the queens of both species, otherwise the dynamics of the population over 6 years of development (total population of the mixed colony from Kyiv: 27 thousand workers of *L. umbratus*, 72 thousand workers - *L. fuliginosus* and two colonies with same population from Rivne) is difficult to explain. The possibility of such mixed colonies co-existing for at least a few years is debated.

Keywords: *Lasius umbratus*, *L. fuliginosus*, mixed colonies, mathematical modelling, trails.

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Introduction

Ants of different species usually inhabit separate nests. However, there are some exceptions. A rudimentary form of symbiosis, plesiobiosis, involves finding nests of different species in close proximity to each other (Czechowski, 2004). The nests themselves exist as separate units and their populations do not mix, i.e. there may be no direct interaction between the species.

Plesiobiosis may be promoted by lack of nesting sites (between *Lasius niger* (Linnaeus, 1758) and *L. flavus* (Fabricius, 1782), *L. platythorax* Seifert, 1991 and *Myrmica rubra* (Linnaeus, 1758)).

A peculiar kind of commensalism was described by Ito *et al.* (2004) in one of the species of the genus *Camponotus* Mayr, 1861 (in the article given as *Camponotus* sp.).

Guided by trace pheromones, *Camponotus* sp. foragers gain access to the food residues of *Crematogaster inflata* Smith, F., 1857, and feed on these residues without causing damage to the "host" species. According to the researchers, the success of *Camponotus* sp. contributes to its similarity with the "host" species in colour (Batesian mimicry). *Crematogaster inflata* has always tried to attack *Camponotus* sp. This suggests that *Camponotus* sp. is a commensal with *C. inflata*, and the similarity of coloration does not help to avoid the attack of *C. inflata*.

Some species use the trails of the "host" species to gain access to its food resources. Such relationships have been described for *Gnamptogenys menadensis* (Mayr, 1877) and *Polyrhachis rufipes* Smith, F., 1858 (Gobin *et al.*, 1998); *Camponotus lateralis* (Olivier, 1792) and *Crematogaster scutellaris* (Olivier, 1792) (Menzel and Blüthgen, 2010), and *C. lateralis* and *Crematogaster schmidtii* (Mayr, 1853) (Stukalyuk and Radchenko, 2011). As for *Camponotus lateralis* and *Crematogaster scutellaris*, *Crematogaster* workers always attack *Camponotus lateralis* workers, but are less aggressive towards workers using the same trail as *Crematogaster* (Menzel and Blüthgen, 2010). *C. lateralis* and *Crematogaster schmidtii* also shows similar behaviors- *Crematogaster* attacks them, while *C. lateralis* avoids aggression (Stukalyuk and Radchenko, 2011). Another variant of coexistence of ants within the same nest is social parasitism.

The phenomenon of parasitism is quite widely and variously represented among ants: from temporary social parasitism, characteristic of red wood ants, as well as some representatives of the genus *Lasius*, to real social parasitism ("slavery" - in *Polyergus rufescens* (Latreille, 1798), *Strongylognathus* spp.). Some ants are obligate kleptoparasites (*Solenopsis fugax* (Latreille, 1798)) (Radchenko, 2016; Seifert, 2018).

Temporary social parasites establish their colonies at the expense of the colony of the "host" species, when their females enter the nests and kill the local queen. These include *Lasius umbratus* (Nylander, 1846), a parasite of *Lasius niger*, and the superparasite species *Lasius fuliginosus* (Latreille, 1798), whose colonies appear and grow, in turn, mainly at the expense of the parasite *L.*

umbratus. *L. fuliginosus* is the only known superparasite species for Europe (Seifert, 2018). B. Seifert (2018) described cases of finding such mixed colonies consisting of *L. fuliginosus* and a second species: *L. umbratus* (7 colonies), *L. sabularum* (Bondroit, 1918) (2), *L. jensi* Seifert, 1982 (2), *L. meridionalis* (Bondroit, 1920) (1) and *L. bicornis* (Foerster, 1850) (1). The number of species on which *L. fuliginosus* parasitizes is quite large, even in laboratory conditions the development of colonies of this species is possible due to *L. niger*.

Both species (*L. fuliginosus* and *L. umbratus*) are characterized by similar and diametrically opposite features of biology: a) large colony size (tens to hundreds of thousands of workers); b) construction of cardboard nests (mainly at the base of trees); c) different tiers of habitat and foraging, *L. umbratus* - a geobiont that rarely appears on the surface of the soil, *L. fuliginosus* - a dendrobiont that has trails on the surface of the soil and on tree trunks; d) both species use the odorous secretion of the mandibular glands in defense or attack (citronellol in *L. umbratus* (Blum *et al.*, 1968), and dendrolasine in *L. fuliginosus* (Bernardi *et al.*, 1967)).

The phenomenon of temporary parasitism in these two species in some cases, as our observations have shown, can develop into co-existence. One of the authors (SS) in 2015 discovered a mixed colony of *L. umbratus* and *L. fuliginosus* on the territory of Kyiv (Ukraine) and recorded workers of both species on the ground, on the trail and in the territory of the forage area. One of the first observations was made back in 1897 by the British scientist Donisthorpe (1915), who observed a mixed colony of two species and their workers entering and exiting the same passages. This phenomenon was described in more detail by the Dutch scientist Lodeizen (1946), who observed such a mixed colony for three years (1939-1941). Subsequently, the mixed colonies *L. umbratus* and *L. fuliginosus* were observed by the Polish myrmecologists B. Pisarski, and, later, W. Czechowski (W. Czechowski, personal communication). Thus, the very existence of mixed colonies of *L. umbratus* and *L. fuliginosus* is possible, although it is observed in isolated cases. Given that *L. fuliginosus* is a parasite of *L. umbratus*, the existence of such mixed colonies is a natural phenomenon. However, this existence

of two species in the same nest is short-lived – after the murder of the *L. umbratus* queen, only one generation of workers of this species, possibly another generation of them, hatched from eggs laid before the murder of the *L. umbratus* queen.

In favor of the option of a longer coexistence of mixed colonies of two species, with the preservation of their queens, we give several arguments.

1. The presence of two species of workers on the trails passing on the surface. We have observed hundreds of *L. fuliginosus* colonies, but no *L. umbratus* workers have ever been identified on their trails, for which access to the surface is excluded during all periods of the life cycle, except during the mating summer.

2. Workers of both species in the nest. The penetration of the queens of *L. fuliginosus* is unlikely in the colonies of *L. umbratus*, numbering tens to hundreds of thousands of workers, since it becomes almost impossible to find the queen of the host species and destroy it. In addition, according to some data, oligogyny is possible in *L. umbratus* (Seifert, 2018), so after the murder of one queen of this species, another may well persist.

3. If the population of both species in the colony is large and numbers tens of thousands – hundreds of thousands, in order to reach the number of workers comparable to *L. umbratus*, the queen (or queens) of *L. fuliginosus* will need more than one year to increase to such a population. The presence of observations of workers of two species in the same colony for 2 years or more (i.e. longer than the life cycle of workers of one generation) will allow us to confidently assert the fact of the preservation of queens of both species within the same colony.

All these assumptions, as well as the data of other researchers, allowed us to assume that the existence of mixed colonies of the two species is possible. This article consists of three parts - biological, which describes the features of the biology of mixed colonies that we have established; mathematical, which analyzes the models of population growth of two species within one mixed colony, and chemical, which analyzes the literature data on pheromones (trace and alarm) used by the two species. According to the authors of this article, trace pheromones played a crucial role in the possibility of co-existence of these two

species when their workers moved together on the trails. Therefore, it is necessary to analyze the chemical structure and proximity of the trace pheromones of *L. umbratus* and *L. fuliginosus* by chemical nomenclature, and compare them with those of other ant species of the genus *Lasius* Fabricius, 1804.

The aim of this study is to describe the case of two mixed colonies discovered by the authors: *L. umbratus* and *L. fuliginosus*. The tasks set by the authors included: a) to study the characteristics of the activity of workers on the trail and forage; b) to identify whether both species live together, in the same nest, or coexist in two different nests (plesiobiosis); c) to analyze the literature data on the chemical composition of trace pheromones in *L. umbratus* and *L. fuliginosus* and other ant species for the similarity of their structure.

Materials and Methods

Nest details

Observations were made on a single mixed colony, *L. umbratus* and *L. fuliginosus*, containing workers of both species on trails and inside the nest. The colony was discovered in the territory of Kyiv in July, 2015. The place of discovery is Zhukov Island (50°21.768 N, 30°33.731 E), located on the southern outskirts of the city. Meadow communities predominate, as well as floodplain forests, often with a high level of shading formed by *Acer negundo* undergrowth. The first tier consists of trees of oak petiolate (*Quercus robur*), black poplar (*Populus nigra*), and white poplar (*Populus alba*), the second tier consists of elm trees (*Ulmus laevis*) and maple undergrowth (*Acer negundo*). The colony lived in the trunk of an old oak tree (1m in diameter, Fig. 1A), the nest and its exits were located under the base of the trunk (Fig. 1B). Along the trunk a forage trail of both species were observed. The neighboring trees were not occupied by these ants. The oak grows on the outskirts of the clearing, at the entrance from the dam to the territory of Zhukov Island, in a recreation zone.

On the second visit in May 2018, the colony could not be found. The 4 colonies of *L. fuliginosus* that are located nearby and the distance to them are taken into account. The data collected in 2015 were used in processing of the obtained material.

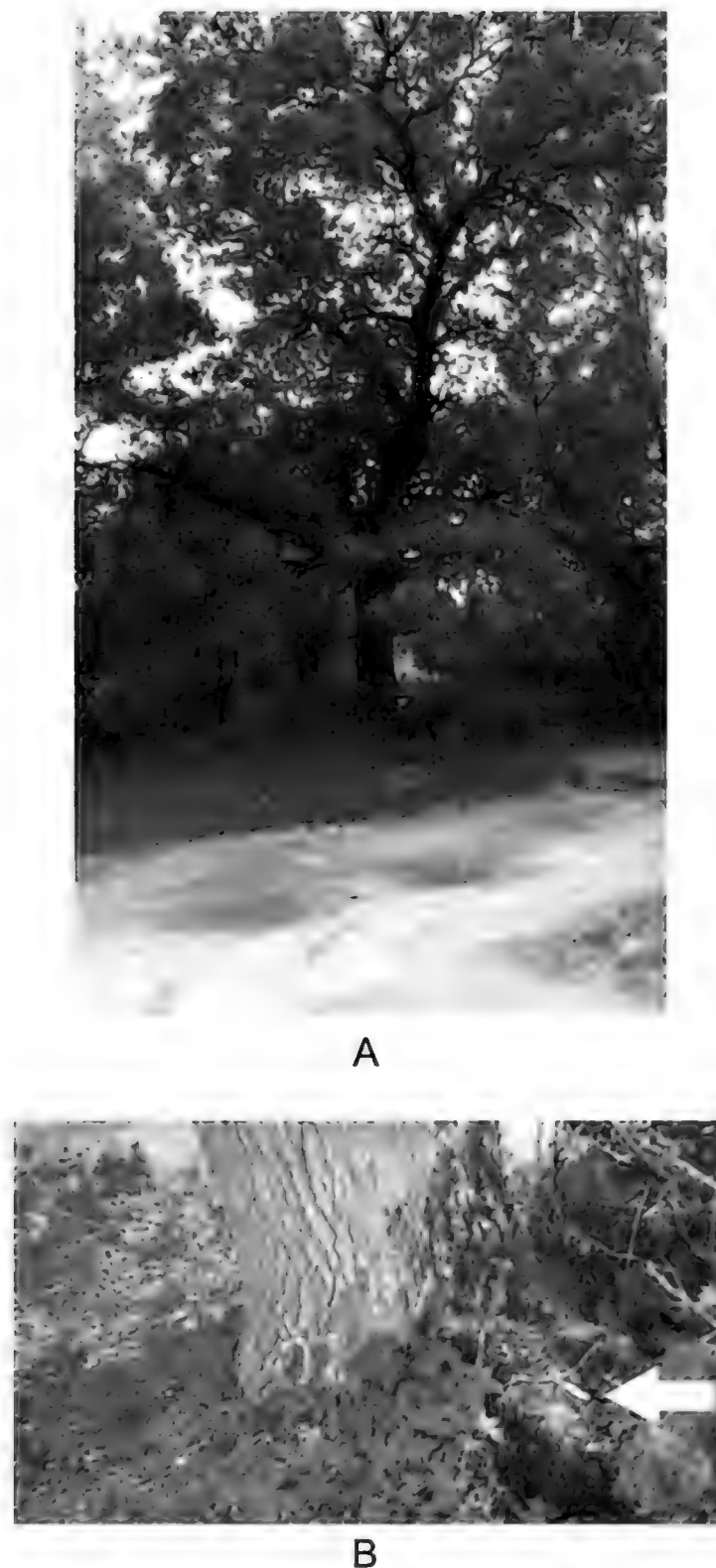


Figure 1. A: *Quercus robur* tree, populated by ants of mixed colony in Kyiv; **B:** nest entrance (marked by arrow).

The second colony was located in the vicinity of Rybnitsa (coordinates 47°65.0982 N, 29°09.8723 E, Moldova, Transnistria), on the territory of a dacha plot. In this colony, the population of *L. fuliginosus* workers was smaller, and the trail was mostly underground. The length of the trail (which was established by excavation) was 26m. The mixed colony was observed for 5 years, from 2012 to 2017, i.e. during this period, at least 4 generations of workers of both species should have changed in it. Unfortunately, we did not take into account the total population of the colony, but according to approximate estimates, there are about 30 thousand *L. umbratus* workers and about 40 thousand of *L. fuliginosus* workers.

In July 2021, in the territory of the city of Rivne (Ukraine), observations were carried out over two more mixed colonies. The first of the colonies (coordinates 50°61.37522 N,

26°30.27270 E) had one trail, and ants from it visited 1 birch tree (*Betula pendula*), at the base of which their nest was located (trunk diameter 0.48m). The second colony was larger, and ants from it controlled 3 birches (trunk diameter 0.6–0.7m), at the base of two of which there were nest entrances (colony coordinates 50°60.9631 N, 26°30.8069 E). There were 2 trails between the trees.

Trails were also found on the trunk of each of the trees in both colonies. Both colonies, as observed by one of the authors (AS) of the article, were at least 4 years old.

Research methods

Antennal contacts on the trail: A video file with ants of both species moving along the trail, lasting 32 seconds, was processed for further statistical analysis. A video record was made (for a period of 2 seconds) of the contacts of all ants that moved along the trail:

the number of antennal contacts among workers of same species as well as between two different species. To do this, we studied the trajectory of each of the ants moving along the trail, the number of contacts with individuals of their own species, with individuals of another species. Ants that moved on trails from aphid feeding grounds were taken into account - they had a swollen abdomen. This is important because it is possible to assume the role of workers of each species on the trail leading to the aphid colonies. In general, the analysis of the file takes into account the trajectories of movement of 16 *L. umbratus* workers and 21 *L. fuliginosus* workers. The total number of 2-second counts was 117 for *L. umbratus*, 99 for *L. fuliginosus*.

Similar calculations were made for two colonies from the city of Rivne. For this, in each of the two colonies, 4 videos were filmed, 3 minutes long, for trails on the ground, and the same number for the trails on tree trunks. For the trails passing on the ground, the trajectories of 135 *L. fuliginosus* workers and 18 *L. umbratus* workers were taken into account. 855 counts per 2 seconds for *L. fuliginosus*, 225 counts for *L. umbratus* were performed. For the trails passing along tree trunks, the trajectories of 57 *L. fuliginosus* workers (405 counts) and 10 *L. umbratus* (64 counts) workers were taken into account, respectively.

Three groups of parameters were designated, among which statistical comparisons were made: a) antennal contact (*umbratus-umbratus*, *umbratus-fuliginosus*, *fuliginosus-fuliginosus*); b) the total number of ants of each species that were in the field of view for 2 seconds of recording; c) the number of ants of each species that did not participate in antenna contact.

Activity on the trails: For two colonies from the city of Rivne, we carried out detailed surveys of workers' activity. This is necessary to establish the ratio of the number of workers of both species of ants on the trails. In addition, on the basis of these data, it becomes possible to calculate the number of foragers in the colony, as well as the total population of the colony.

The counts were carried out at 7.00 PM local time, when the ants were most active, after the air temperature dropped to 26-27°C. Each count of activity was carried out

for two minutes. All ants of both species passing under a gate-shaped barrier above the trail were counted. They did not put a gate on the tree, but chose a place on the trunk, along which it was easy to navigate (a branch, a crack in the bark). For both colonies, 30 counts each were made for a trail on the tree and for a trail on the ground.

Foraging activity: To check the foraging activity of both ant species, baits were laid out at an equal distance (0.5 m) along the trail on the ground in each of the two colonies. Preliminarily, killed individuals of the Turkmen cockroach (*Shelfordella tartara*) were used as bait. In total, 60 baits were laid out, 30 in each colony. The counts of the number of ants of both species were kept until the bait (1 cockroach = 1 bait) was moved to the nest. For colony 1, this time was 1 hour (6 counts of each bait, 180 counts in total), for colony 2 - 30 minutes (3 counts of each bait, 90 counts in total).

Protection of the nest: To check the participation of both species in the protection of the nest, in colony 2, 100 pre-collected workers of *Lasius niger* were planted to the nest entrance. A video was filmed (duration 12 minutes), during the processing of which the number of *L. fuliginosus*, *L. umbratus* ants that came out to defend the nest, the number of their attacks against *L. niger* workers for each 1 minute of video was recorded.

Excavation of the nest: For both colonies from the city of Rivne, excavations were carried out in order to take into account the approximate ratio of ants of both species inside the nest. Excavations were carried out to the level of the cardboard part of the nest.

Calculations of the number of foragers and the total worker population in the mixed colony: The number of foragers, as well as the total number of workers of the two species in the nest, was established using the formula proposed by Zakharov (1978, 2015). Foragers make up an average of 13% of the colony population, so it is possible to calculate the colony population for each of the species.

$$A = 36.82 - 2.127 I + 0.112 I^2 - 0.00047 I^3 \quad (1a)$$

A— the number of foragers (in hundreds of individuals); I— the number of workers (individuals per 1 minute on the trail). The accuracy of the method is $P = \pm 1.6\%$.

To calculate the number of workers, 3 observations of the trail for 5 minutes were sufficient, while taking into account the number of workers passing in one direction along the trail. Formula 1b was used for calculation of the total population of the colony:

$$N = 7.7A \quad (1b)$$

Number of foragers on average should be equal to 12.98% (13%) of the total number of workers in the colony (Zakharov, 2015). These data agree with others, according to which the number of foragers in *L. fuliginosus* may be 15% of the total population (Hennaut-Riche *et al.*, 1980)

Mathematical modeling

In order to create a mathematical model, we have made assumptions that are based on the literature data.

1. One queen could support a population of up to 60 thousand workers at a time. These data are given for *L. niger* (Boomsma *et al.*, 1982), however, we did not find data for *L. umbratus*, *L. fuliginosus*. Taking into account the fact that all species have physogastric queens, but the colony population of *L. umbratus*, *L. fuliginosus* is on average 2-10 times larger (on average, 13 thousand in *L. niger*, 20-30 - *L. umbratus*, 100 thousand - 1 million in *L. fuliginosus* (Radchenko, 2016) or even up to 5 million (Zakharov, 2015)), we assume that the queens of these species are more fertile, and, consequently, faster rates of colony growth in *L. umbratus*, *L. fuliginosus* compared to *L. niger*. By knowing the total population of a colony, we can guess how many queens of each species are there, provided the population that each queen is able to support is already known. For *L. umbratus*, we leave the figure at 60 thousand, because on average, its colonies are slightly larger than those of *L. niger*, for *L. fuliginosus*, we allow a figure of 100 thousand and the growth rate is 5 times higher compared to *L. umbratus*. *L. umbratus*, in turn, develops 5 times more intensively than *L. niger*, since its queen (as well as the queen of *L. fuliginosus*), begins oviposition not at the expense of its own reserves of the fat body, but at the expense of a full-fledged abundant nutrition in the already formed colony of the host species.

2. The population in a mixed colony (calculated according to the Zakharov

formula) was 72.816 workers of *L. fuliginosus* (of which 9.466 are foragers) and 27.740 workers of *L. umbratus* (3.606 foragers). For colony 1 from Rivne, the population was 53.651 workers of *L. fuliginosus* (6.967 foragers) and 24.261 workers of *L. umbratus* (3.151 foragers). For colony 2 from Rivne, the population was 65.043 workers of *L. fuliginosus* (8.447 foragers) and 22.607 workers of *L. umbratus* (2.935 foragers).

3. At the time of the penetration of the queen of *L. fuliginosus* into the colony of *L. umbratus*, there were about 5 thousand workers. We believe that, invasion of small colonies of *L. umbratus* is unlikely, since the mass oviposition of *L. fuliginosus* requires an abundant supply of food, and consequently, a large number of foragers of *L. umbratus* collecting it. Therefore, we chose the number of *L. umbratus* workers corresponding to the average number of workers in the colony.

4. The period of egg laying for two species is from spring (end of March) to August inclusive, by analogy with *L. niger* (Kipyatkov, 2007).

5. The life expectancy of the queen in both species is from 5 years to 20 years and the worker - one season. Workers come out of the cocoons in June and can live until the next July-August. Therefore, there is a change of generations, and each subsequent one should exceed the number of the previous one. The exact life span of a worker is taken by analogy with the data for *L. niger*, whose workers on average live a little more than a year – 434 days in young colonies and 309 days in an adult colony (Kramer *et al.*, 2016). The maximum life expectancy of workers was 1094-1129 days, but less than 1% of workers live to this age. The majority of workers (95 %) die out the very next year after birth.

6. From eggs laid in the summer, a generation of workers comes out in the fall, if the foundation occurred in June, if later - then in May-June of the following year. It is only one generation of workers per year (Seifert, 2018).

7. The rate of population growth is the same for the two species, but different depending on the age of the colony. In the laboratory colonies of *L. niger*, it is 300, 1500 and 5000 workers for one, two and three years after the foundation of the colony (Buschinger, 2016). Further growth we built on this trend, taking into account the specific feature (greater

fecundity) of *L. umbratus* and *L. fuliginosus*. In addition, we used data on the growth rate of honeybee colonies (*Apis mellifera* L.), which increased their number from 6 thousand workers to 15 thousand in 120 days (Rangel *et al.*, 2013).

8. From the moment of the penetration and murder of the queen of *L. umbratus* (or a failed murder attempt, and preservation of the queens of both species), the oviposition of *L. fuliginosus* begins only after 5-14 days (Seifert, 2018). Penetration occurs after the mating summer, that is, from the beginning of June-July.

When modeling the behavior (development) of living systems, the question arises about the method by which this could be achieved. Fortunately, despite the diversity of living systems, they have specific features. This allows us to develop common approaches for the construction of such models. As a rule, models take into account the following:

- biological systems are complex multicomponent, spatially structured, the elements of which have individuality;
- these are multiplying systems (capable of autoreproduction);
- they are open systems that constantly pass through the flow of matter and energy;
- biological objects have a complex multi-level system of regulation.

Conditionally, all mathematical models of biological systems can be divided into regression, qualitative, and simulation models.

Currently, due to the development of computing and graphics capabilities of computers, simulation models play an increasingly important role.

The essence of simulation modeling is to study a complex mathematical model using computational experiments and processing the results of these experiments.

The process of building a simulation model is as follows. In a formalized form accessible to the computer (in the form of equations, graphs, logical relations), everything known about the system is set. Then the options are played on the computer for what the totality of this knowledge can give for certain values of the external and internal parameters of the system.

Simulation models of specific living systems take into account the available information about the object as much as

possible. When creating a simulation model, you can afford a high degree of detail when selecting the variables and parameters of the model. At the same time, the model may be different for different authors, since there are no exact formal rules for its construction. The results of machine experiments depend not only on the relations inherent in the model, but also on the organization of the complex programs implementing the model, and on the mechanism of conducting machine experiments (Riznichenko, 2002).

In our case, the main tasks of simulation modeling were:

- * testing the hypothesis about the interaction of individual ant species;
- * forecast of their behavior when changing internal characteristics (terms of invasion, its conditions);

When constructing a simulation model of the process of coexistence of colonies of *L. fuliginosus* and *L. umbratus*, it is considered that the population size changes over time (grow, fall, possibly fluctuate) due to the changes in the productivity of the queen of the corresponding species.

In our case, this is a systemic factor that limits the growth of the population.

In the classical models of mathematical ecology, this phenomenon is described by the equation of logistic growth (Riznichenko, 2002):

$$\frac{\partial x}{\partial \tau} = r \cdot x \cdot \left(1 - \frac{x}{K}\right) \quad (2)$$

x – colony size;

τ – time;

r – growth rate constant;

K is the population capacity.

This equation has an analytical solution:

$$X(\tau) = \frac{X_0 \cdot K \cdot e^{r\tau}}{K - X_0 + X_0 \cdot e^{r\tau}} \quad (3)$$

X_0 – initial number of specimens.

This solution demonstrates two features of growth: at small values of X , the number increases exponentially, at large values, it approaches a certain limit of K .

Formula (3) describes the kinetic curve, that is, the dependence of the population size on time. In contrast to (3), assuming that the number of eggs $Y(x)$ that the queen lays does not depend on time, but on the number of worker ants of a given species, we

obtained an expression for calculating her productivity over a weekly cycle:

$$Y(x) = \frac{Y_0 \cdot K \cdot e^{rx}}{K - Y_0 + Y_0 \cdot e^{rx}} \quad (4)$$

Y_0 – initial productivity.

The survival rate of the pupae is also determined by the number of workers of this species. The total population of the anthill of a species has been calculated taking into account the average life expectancy of a working ant equal to 400 days, given a mortality percentage equal to 10% of the number of overwintered ones.

As part of our work, we considered the following options, or variants of development:

1 – 1 queen of *L. umbratus* was killed and 1 queen of *L. fuliginosus* settled;

2 – both queens are alive after the introduction of 1 queen of *L. fuliginosus*;

3 – 1 of the 2 *L. umbratus* queen is alive, 2 queens of *L. fuliginosus* have been introduced;

4 – two queens of *L. umbratus* are alive after the introduction of 2 *L. fuliginosus* queens.

Only 1 out of these 4 options provide for the presence of two queens in both species. Only two colonies of *L. fuliginosus* out of 33 surveyed in Germany had more than one queen (Elst and Gadau, 2018). According to other data, *L. fuliginosus* might have several queens (Czechowski *et al.*, 2012). *L. umbratus* is mainly characterized by a single queen, but oligogyny is also possible (Seifert, 2018). All this serves as a basis for considering not only options with monogyny, but also with oligogyny.

Statistical analysis

Statistical calculations were performed with the Past v. 4.03 software. The data were checked for the normality of the distribution, in case of non-compliance with the normal distribution, nonparametric analysis methods were used. Differences between groups (the number of antennal contacts, between one species and both species; the average number of ants of each species for 2 seconds of accounting; the number of ants of both species that did not participate in antennal contact) were checked using the Kruskal-Wallis test (K-W) for equal medians. In the case of significant differences, the Mann-Whitney test (M-W, with Bonferroni corrected p value) was used. The average values of the parameters are

shown in the form of Barchart-boxplot (Past v. 4.03), the mode of violin and box.

Results and Discussion

Aft section: For the mixed colony in Kyiv, it includes only one tree and one trail, which is less than the average for *L. fuliginosus* from the same area, in the territory of mixed forests (Stukalyuk, 2017). On an average, there are 3.4 ± 0.2 trails per single-species colony of *L. fuliginosus*, 13.0 ± 2.3 trees visited by ants. The activity of ants on the trails averages 50.7 ± 6.5 workers per 2 min for 2015, with maximum values up to 150-200 workers per 2 min. For a mixed colony in Kyiv, this indicator was 87.0 ± 9.6 workers of *L. fuliginosus* and 19.6 ± 4.8 workers of *L. umbratus* for 2 minutes of accounting. For colony 1 from the city of Rivne, these parameters were 64.9 ± 1.4 *L. fuliginosus* and 5.9 ± 0.42 *L. umbratus* workers; for colony 2: 72.0 ± 3.1 *L. fuliginosus* and 9.2 ± 0.7 *L. umbratus* workers, respectively. Thus, the level of the activity of *L. fuliginosus* workers on the trails in a mixed colony is different from single-species ones. The workers of *L. umbratus* on the trails were more than 5.0 times less numerous than the workers of *L. fuliginosus* (at $p < 0.05$). The expansion (and later, possibly, relocation) of the colony could be prevented by the open spaces between neighboring trees, which are trampled earth.

The death of mixed colonies in Kyiv and in Transnistria: A second inspection in 2018 showed that the colony died, possibly more than a year ago. The oak was occupied and visited by other ant species - *Lasius brunneus* (Latreille, 1798), *Camponotus fallax* (Nylander, 1856), *Dolichoderus quadripunctatus* (Linnaeus, 1771). The excavations revealed the abandoned remains of cardboard structures, but without remains of the bodies of the dead ants.

The nearest colonies of *Lasius fuliginosus* were located at distances of 300-700m, the closest at 150m, separated by a road and had a size of an order of larger magnitude. Most likely, a mixed colony of both species, for some reason, died. Nearby solitary oaks were occupied by colonies of *Lasius brunneus*, sometimes *Camponotus fallax*, *Dolichoderus quadripunctatus*. The trees of other species were dominated by *Lasius niger*.

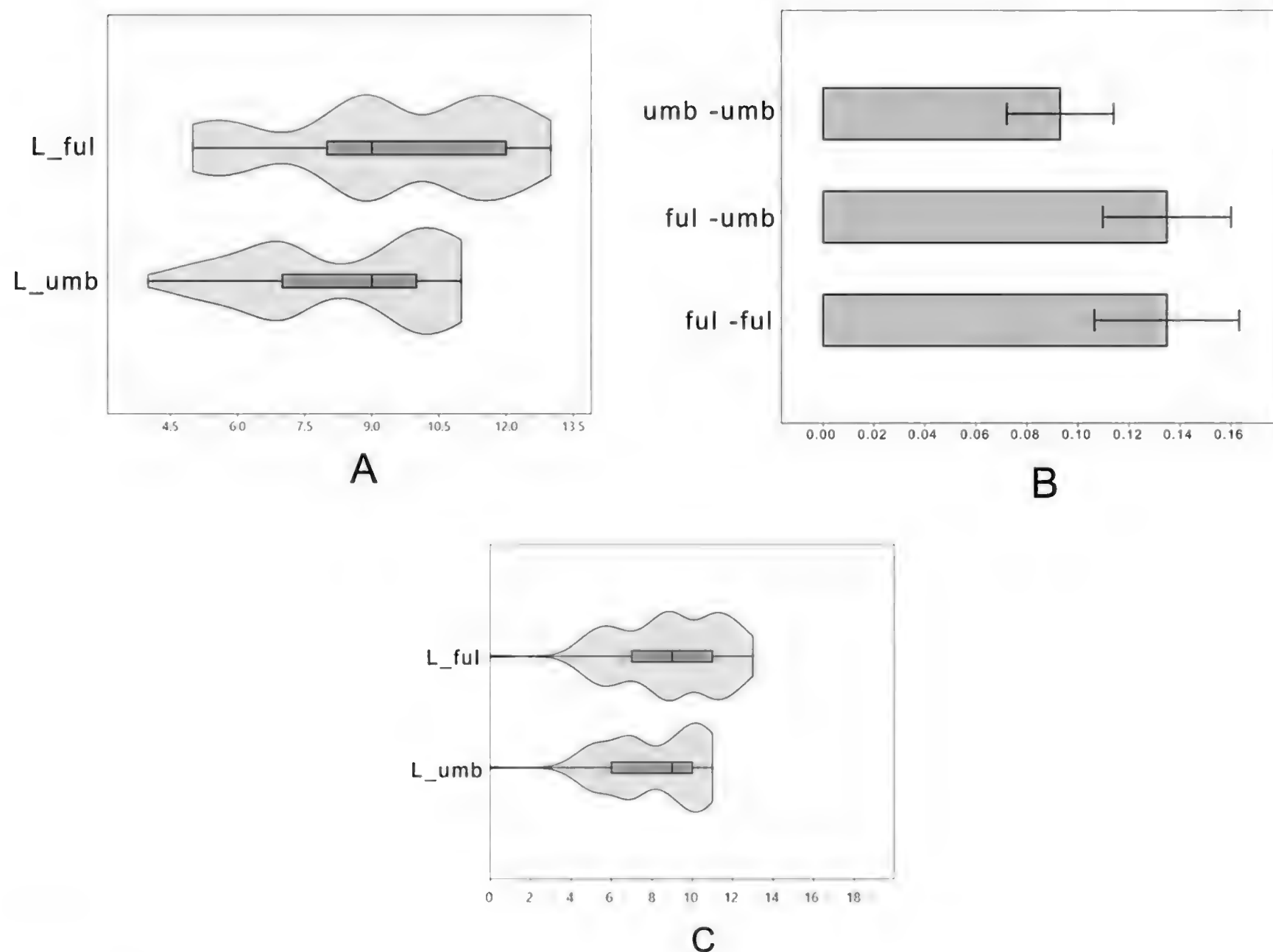


Figure 2. **A:** The average number of workers of *Lasius umbratus*, *L. fuliginosus* for 2 seconds of accounting on the trail of a mixed colony (Kyiv); **B:** The average number of workers of *Lasius umbratus*, *L. fuliginosus* for 2 seconds of accounting, who participated in antennal contact on the trail of a mixed colony (Kyiv); **C:** The average number of workers of *Lasius umbratus*, *L. fuliginosus* for 2 seconds of accounting, who did not participate in antennal contact on the trail of a mixed colony (Kyiv).

The mixed colony from Transnistria also died, both species disappeared. This was consistent with the data for Kyiv and indicates that if both queens are preserved, the colony will most likely don't last longer than 5-7 years.

A mixed colony may break up due to the death of one of the queens. The colony we studied could have appeared under the following circumstances: when the queen of *L. fuliginosus* entered, the queen of *L. umbratus* was not killed or there might have been several queens in the colony of *L. umbratus*.

Antennal contacts on the trail

Kyiv colony: It was found that on the trail, the average number of workers of both species differed in two seconds of accounting - there were more *L. fuliginosus* workers (K-W: $p = 9,179 \text{ E-}06$) (Fig. 2A). Thus, on the trail, the number of *L. fuliginosus* workers prevails. It may be due to the peculiarities of the

biology of *L. umbratus* - in contrast to *L. fuliginosus*, foraging by *L. umbratus* is carried out underground, not on the surface. The very fact of finding workers of *L. umbratus* on the mixed-colony trail is a very unusual feature of their biology.

There is a positive relationship between the number of workers of both species on the mixed-colony trail (M-W, $p = 9.195 \text{ E-}06$). Workers of both species made antennal contacts, both intraspecific and interspecific (Fig. 2B). At the same time, no significant differences were found between the number of contacts in 2 seconds (K-W: $p = 0.4513$). That is, workers of both species were equally often in contact with individuals of their own species, as well as with individuals of another species.

Significant differences were found between the number of workers of both species who did not participate in antennal contact, but simply moved along the trail of the mixed colony (K-W: $p = 0.0004891$) - the

number of *L. fuliginosus* workers is greater, while the differences are significant (M-W: $p = 0.0004898$) (Fig. 2C).

It follows from this that the number of *L. fuliginosus* workers on the trail is slightly higher than that for *L. umbratus*, and therefore the number of *L. fuliginosus* workers that do not participate in antennal contact is also higher. It is obvious that the duration of the video is too short, so we received updated data on the number of workers of both species after the trail surveys. At the same time, workers of both species are willing to contact both within the same species and interspecific contacts. Such behavior on the trails in a mixed colony of two parasitic species is possible only in the case of a close structure of pheromones-traces, alarm pheromones, etc., or in the case of an exchange of cuticular hydrocarbons.

Rivne colony: Significant differences were established for the number of antennal contacts between workers of two species on a trail passing along the ground (K-W: $p = 9.926E-12$, Fig. 3A). The maximum number of antennal contacts were between workers of *L. fuliginosus*; interspecific contacts are somewhat less intense (M-W: $p = 0.02448$). Contacts between workers of *L. umbratus* are the least frequent, less frequent than interspecific contacts (M-W: $p = 1.864E-06$), and contacts between workers *L. fuliginosus* (M-W: $p = 5.357E-10$).

On the trails passing along the trunk of trees, no antennal contacts between workers of *L. umbratus* were recorded. Differences between the other two categories of contacts are significant (K-W: $p = 0.02547$, Fig. 3B). The number of contacts between workers *L. fuliginosus* is, on an average, greater than the number of interspecific contacts (M-W: $p = 0.02561$). The lower number of *L. umbratus* workers on the trails passing on the ground, and their almost complete absence on the trails passing through tree trunks, is the reason for the low number of intraspecific contacts compared to other categories.

Thus, in a mixed colony, there is a full-fledged coexistence of the workers of the two species, with antennal contacts, following the trails (possibly by the trace pheromones of *L. fuliginosus*). The very fact of the presence of *L. umbratus* workers on the trails outside the nest indicates a change in their lifestyle. They are less visually oriented on the surface, and their eyes contain up to 60 ommatidia

(Blum *et al.*, 1968). Therefore, it can be assumed that the trace pheromones of *L. fuliginosus* for *L. umbratus* will play a key role when moving outside the nest.

Activity on trails

For colony 1 from the city of Rivne, a significant difference was found in the number of ants on the trails passing along the surface of the earth (K-W, $p = 2.019E-20$, Fig. 4A). There are more *L. fuliginosus* workers on the trails than *L. umbratus* workers (M-W, $p = 7.44E-11$, Fig. 4A). In colony 2, the same pattern is observed (M-W, $p = 1.76E-10$). There is no statistically significant difference between the number of *L. fuliginosus* workers on the trails in the two colonies (M-W, $p = 0.4476$). The number of *L. umbratus* workers on the trails is higher in colony 2 than in colony 1 (M-W, $p = 0.002647$, Fig. 4A).

The number of ants on the trails passing along tree trunks also differs (K-W, $p = 3.46E-22$, Fig. 4B). The number of workers of *L. fuliginosus* in colony 1 significantly exceeds the number of workers of *L. umbratus* (M-W, $p = 8.49E-11$, Fig. 4B). In colony 2, the situation is similar (M-W, $p = 1.62E-10$). The number of *L. fuliginosus* workers on the tree trunk trail in colony 2 is slightly higher than in colony 1 (M-W, $p = 1.82E-07$). Similarly, the number of *L. umbratus* ants in colony 2 on the trails passing on the trees is higher than in colony 1 (M-W, $p = 2.03E-05$, Fig. 4B).

Foraging activity

The general tendency for both colonies from the city of Rivne is an increase in the number of *L. fuliginosus* and *L. umbratus* on baits over time (Figs. 4C, D). Moreover, the difference in their number is significant between species (K-W, colony 1, $p = 3.22E-26$; colony 2, $p = 2.28E-50$). An increase in the number of *L. fuliginosus* foragers on baits in colony 1 occurs in the first 30 minutes; in the next 20 minutes, their number slightly decreases and grows again after 1 hour (Table 1). For *L. umbratus*, the number of workers in the first 40 minutes increased 10 times (Fig. 4C), then decreased slightly (50 minutes) and again stabilized at the same level, 60 minutes after laying out the baits. The number of *L. fuliginosus* workers on baits did not increase so significantly, by 4 times (Fig. 4C). For colony 2, the number of workers in *L. umbratus* doubled only after half an hour, while the number of workers in *L. fuliginosus* increased by only half (Fig. 4D).

Mixed colonies of *L. umbratus* & *L. fuliginosus*: when superparasitism may develop into coexistence

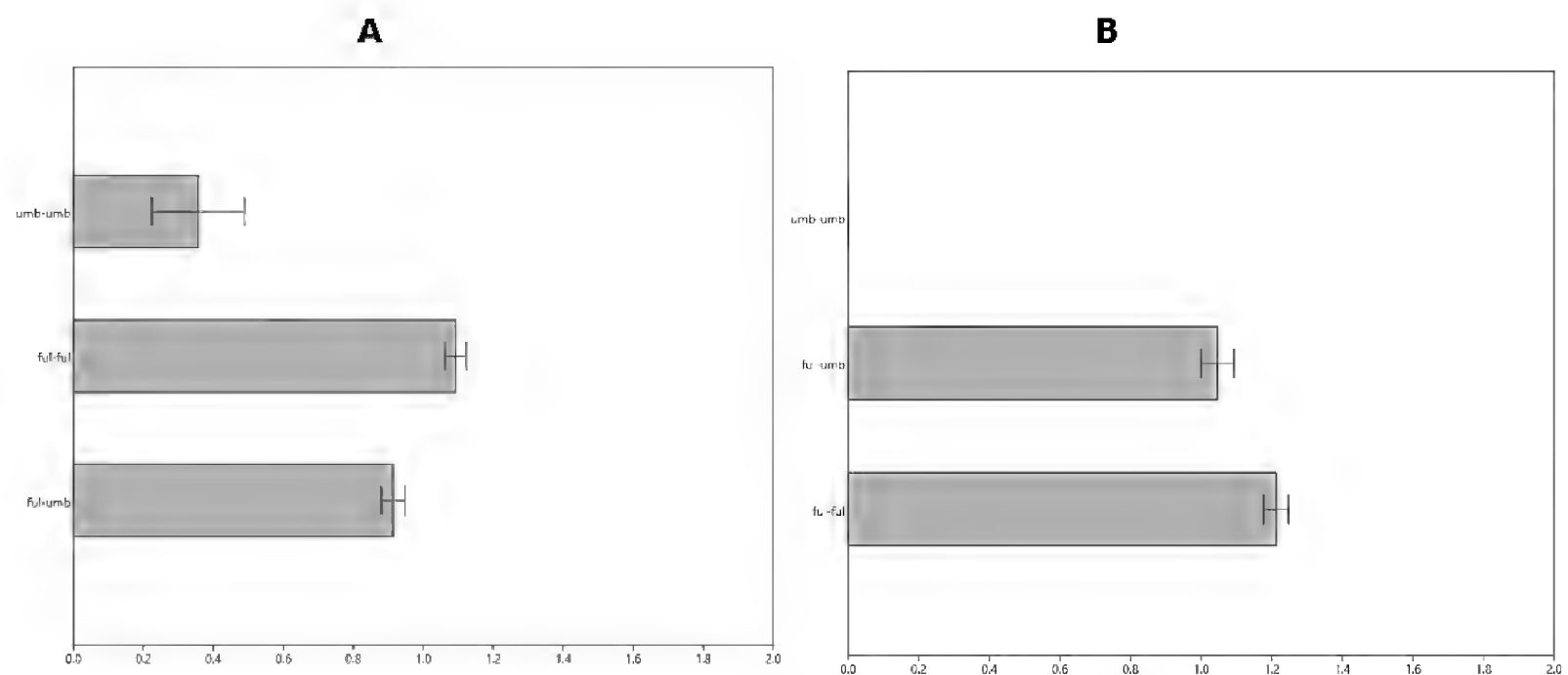


Figure 3. A: The average number of workers of *Lasius umbratus*, *L. fuliginosus* for 2 seconds of accounting, who participate in antennal contact on the trail on the ground of a mixed colony (Rivne); **B:** same, but trail on tree trunk.

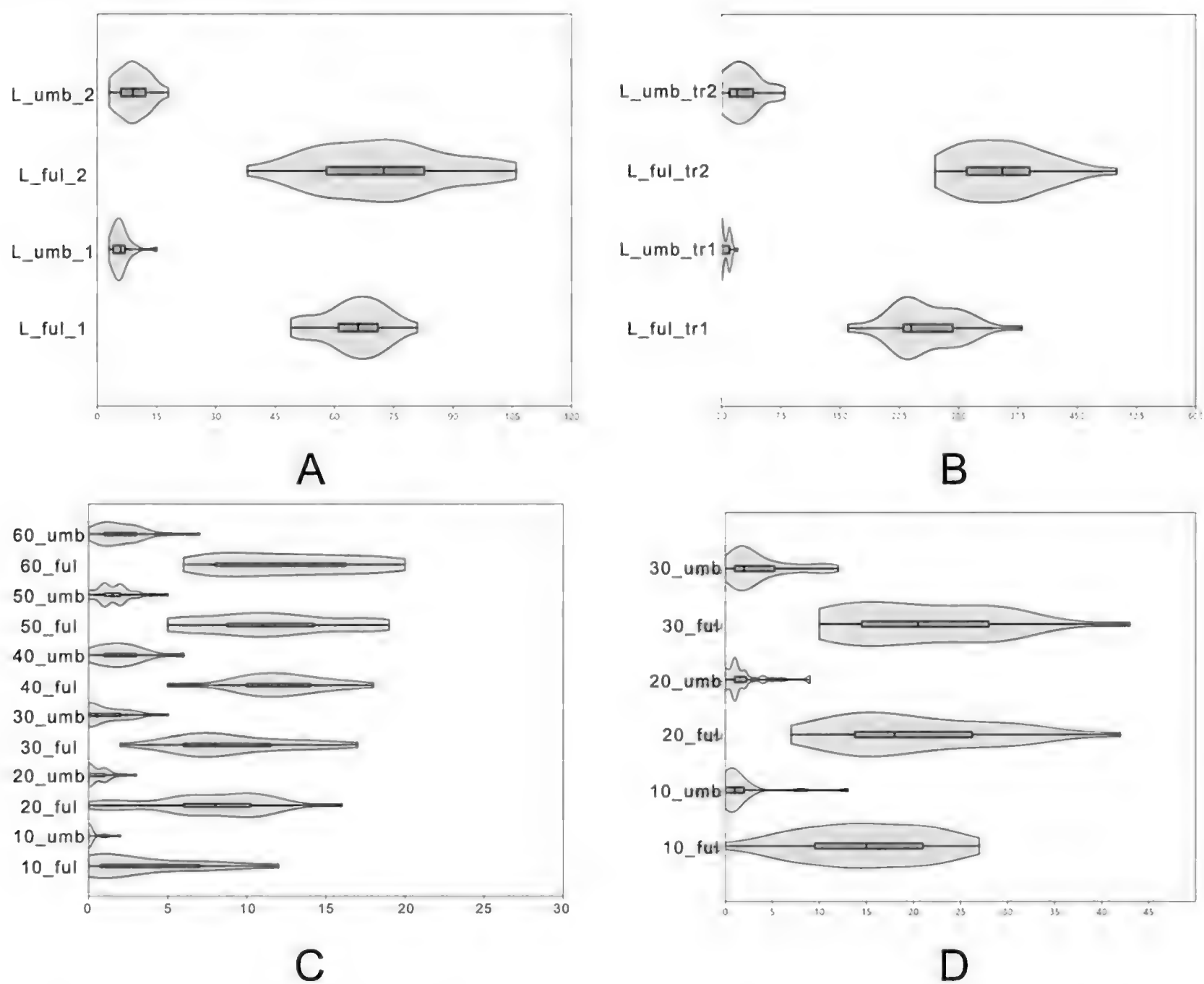


Figure 4. A: Average number of workers *L. fuliginosus*, *L. umbratus* for 2 minutes of counting on the trails passing on the ground in colony 1 (L_ful_1, L_umb_1) and in colony 2 (L_ful_2, L_umb_2), Rivne, Ukraine; **B:** Average number of workers *L. fuliginosus*, *L. umbratus* for 2 minutes of counting on the trails passing on the trunks of trees in colony 1 (L_ful_tr1, L_umb_tr1) and in colony 2 (L_ful_tr2, L_umb_tr2), Rivne, Ukraine; **C:** Average number of workers *L. fuliginosus*, *L. umbratus* attracted on bait in colony 1 during 10 (10_ful, 10_umb), 20 (20_ful, 20_umb), 30 (30_ful, 30_umb), 40 (40_ful, 40_umb), 50 (50_ful, 50_umb), 60 (60_ful, 60_umb) minutes of accounting, Rivne, Ukraine; **D:** Average number of workers *L. fuliginosus*, *L. umbratus* attracted to bait in colony 2 during 10 (10_ful, 10_umb), 20 (20_ful, 20_umb), 30 (30_ful, 30_umb) minutes of counting., Rivne, Ukraine.



A



B

Figure 5. A: *L. fuliginosus*, *L. umbratus* workers on baits, colony 1; B: colony 2, Rivne, Ukraine.

The intensity of foraging in colony 2 also increased with time; there are significant differences between values of 10-30 minutes for both ant species (Table 2). In both species, the number of workers mobilized for bait, increased over time.

It is worth noting that the workers of *L. umbratus* generally did not so much participate in the transport of the bait, but rather moved alongside it. We noted isolated cases of feeding *L. umbratus* workers on bait in both colonies (Figs. 5A, B). From this, it can be concluded that in the outer territory of the forage area, *L. umbratus* workers generally

simply moves along trails, and does not participate in transporting prey, hunting it, or visiting aphid colonies (the latter according to data obtained for the colony from Kyiv).

Protection of the nest

For workers of all three species, significant differences were found in changes in numbers over time (K-W: $p = 4.339E-07$). As the number of *L. fuliginosus* workers increased, more *L. umbratus* workers were mobilized (M-W: $p = 0.0005339$), and the number of *L. niger* workers fell steadily (M-W: $p = 0.002625$). During the first 2 minutes, the number of *L. niger* workers decreased by 5 times; most of them left the landing site immediately after attacks from *L. fuliginosus* (Fig. 6A). After 7 minutes, all *L. niger* workers either disappeared or were killed. The attacks were mainly initiated by the workers of *L. fuliginosus*, but the workers of *L. umbratus* also attacked the enemy (*L. niger*), albeit at a lower frequency (Fig. 6B). With an increase in the number of *L. fuliginosus* workers, the number of their attacks against *L. niger* increased (MW, $p = 0.006477$), increasing number of attacks by *L. umbratus* against *L. niger* (MW: $p = 0.0003906$) and counter attack by *L. niger* against *L. fuliginosus* and *L. umbratus* (MW: $p = 0.0003351$) were also observed. The increase in the number of *L. umbratus* also contributed to an increase in the frequency of their attacks against *L. niger* (MW: $p = 0.02209$) and response attacks of *L. niger* against *L. umbratus* (MW: $p = 0.01589$). It is interesting to note that the number of *L. fuliginosus* workers, as well as *L. umbratus* workers, remained consistently high almost until the end of the video recording, having only slight decrease in the last 2 minutes. This retention of a high concentration of workers of both species can be interpreted.

Excavation of nests

Excavations of the nests of colonies 1 and 2 (Rivne) showed different results. In colony 1, it was possible to only partially open the layer of wood under which the nest was located. Visually, the ratio of workers of the two species was in favor of *L. fuliginosus*, of which there were about 3 times more (we counted 127 workers of *L. fuliginosus* and 43 workers of *L. umbratus*). In colony 2, we managed to excavate the nest down to the cardboard part.

Mixed colonies of *L. umbratus* & *L. fuliginosus*: when superparasitism may develop into coexistence

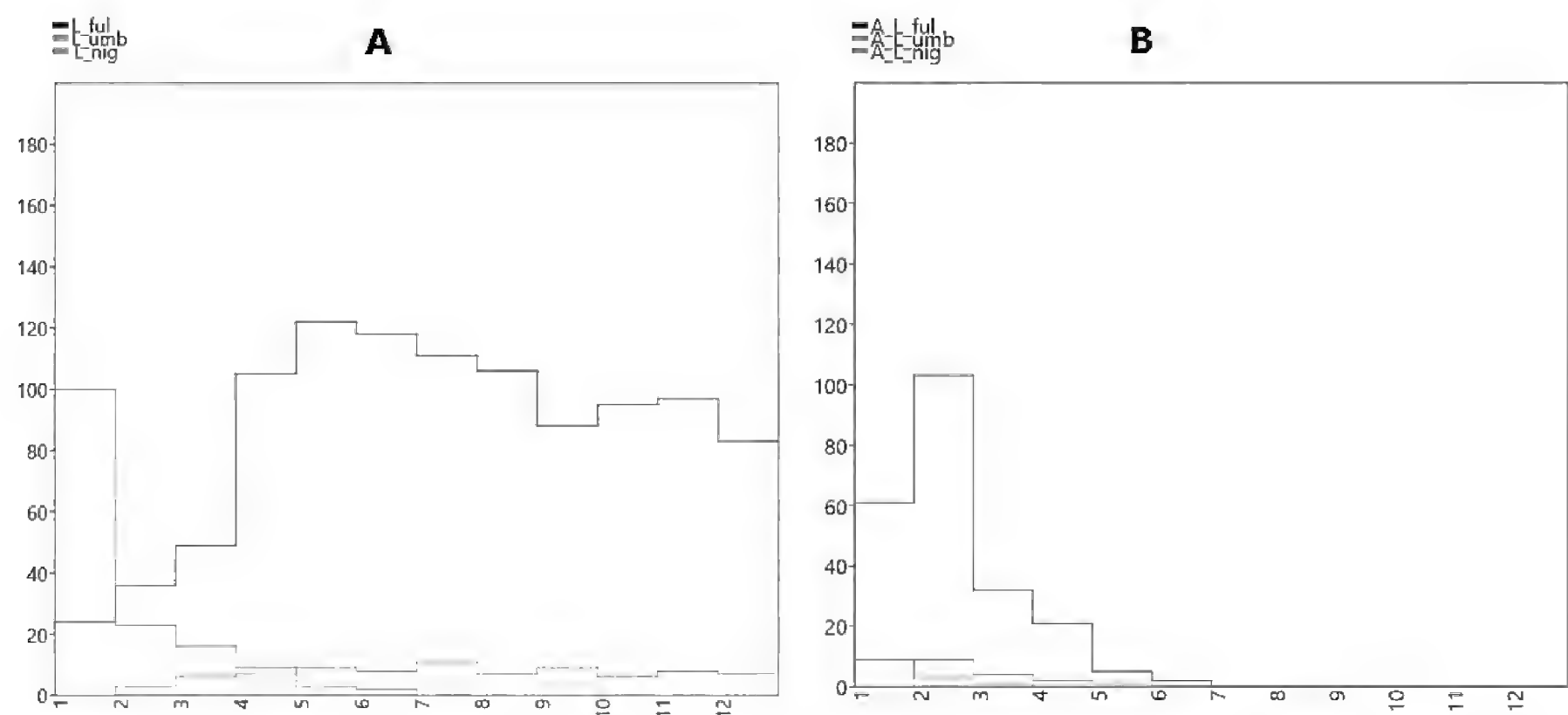


Figure 6. A: Number of workers *L. niger*, *L. fuliginosus*, *L. umbratus* / 1 min at the nest entrance after the appearance of 100 workers *L. niger*; **B:** number of interspecies attacks / 1 min. Vertically - the number of workers (or attacks) / 1 minute; horizontally - time, minutes.

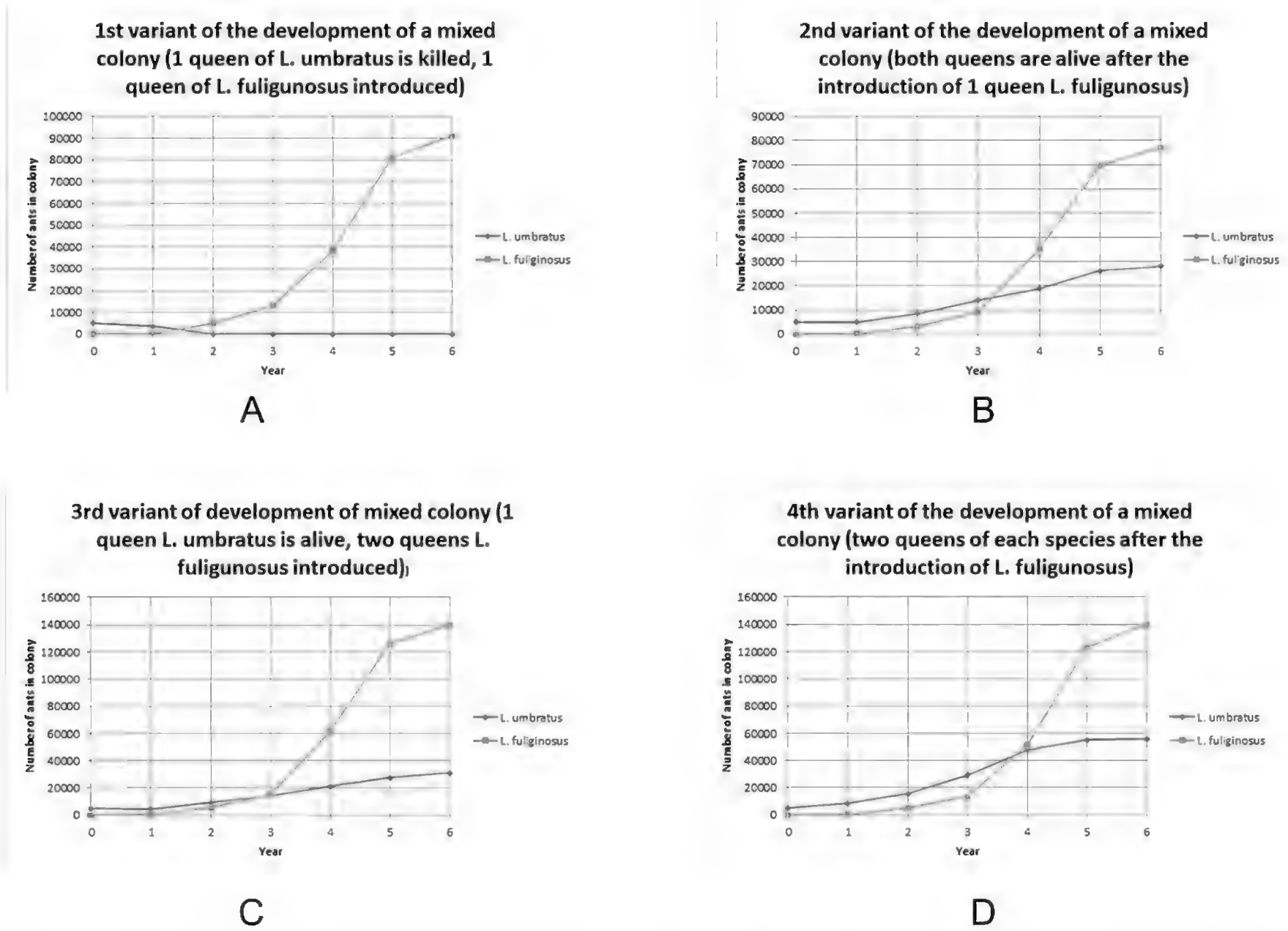


Figure 7. Results of mathematical modelling of the growth of the number of *L. umbratus* and *L. fuliginosus* workers in a mixed colony in four variants over a period of 6 years.

Table 1. Foraging activity in mixed colony 1, Rivne (Ukraine), results of Mann-Whitney pairwise comparison (with Bonferroni corrected p values)

Time of counting and ant species	10_ful	10_umb	20_ful	20_umb	30_ful	30_umb	40_ful	40_umb	50_ful	50_umb	60_ful	60_umb
10_ful		5.63E-05	0.0186	0.01429	0.000174	0.3461	1.64E-07	1	2.73E-06	1	3.54E-07	1
10_umb	5.63E-05		3.42E-09	1	3.44E-10	0.18	3.26E-10	5.55E-06	3.30E-10	3.78E-06	3.33E-10	2.69E-06
20_ful	0.0186	3.42E-09		5.23E-08	1	5.03E-07	0.000699	2.37E-05	0.1158	5.41E-06	0.005579	1.99E-05
20_umb	0.01429	1	5.23E-08		1.24E-09	1	1.02E-09	0.002598	1.02E-09	0.005271	1.03E-09	0.002284
30_ful	0.000174	3.44E-10	1	1.24E-09		3.19E-09	0.115	1.72E-08	1	4.78E-09	0.1386	1.62E-08
30_umb	0.3461	0.18	5.03E-07	1	3.19E-09		1.36E-09	0.6441	1.45E-09	1	1.32E-09	0.7194
40_ful	1.64E-07	3.26E-10	0.000699	1.02E-09	0.115	1.36E-09		2.28E-09	1	1.56E-09	1	2.08E-09
40_umb	1	5.55E-06	2.37E-05	0.002598	1.72E-08	0.6441	2.28E-09		3.74E-09	1	1.90E-09	1
50_ful	2.73E-06	3.30E-10	0.1158	1.02E-09	1	1.45E-09	1	3.74E-09		1.66E-09	1	3.29E-09
50_umb	1	3.78E-06	5.41E-06	0.005271	4.78E-09	1	1.56E-09	1	1.66E-09		1.51E-09	1
60_ful	3.54E-07	3.33E-10	0.005579	1.03E-09	0.1386	1.32E-09	1	1.90E-09	1	1.51E-09		2.01E-09
60_umb	1	2.69E-06	1.99E-05	0.002284	1.62E-08	0.7194	2.08E-09	1	3.29E-09	1	2.01E-09	

Note. The designations in the columns are as in Fig. 4C (counting time, minutes and species of ants), insignificant differences are highlighted in bold.

Table 2. Foraging activity in mixed colony 2, Rivne (Ukraine), results of Mann-Whitney pairwise comparison (with Bonferroni corrected p values)

Time of counting and ant species	10_ful	10_umb	20_ful	20_umb	30_ful	30_umb
10_ful		1,71E-08	0,4945	1,99E-08	0,1013	3,03E-07
10_umb	1,71E-08		7,36E-10	1	6,35E-10	0,005434
20_ful	0,4945	7,36E-10		5,96E-10	1	2,19E-09
20_umb	1,99E-08	1	5,96E-10		3,62E-10	0,1676
30_ful	0,1013	6,35E-10	1	3,62E-10		1,21E-09
30_umb	3,03E-07	0,005434	2,19E-09	0,1676	1,21E-09	

Note. The designations in the columns are as in Fig. 4D (counting time, minutes and species of ants), insignificant differences are highlighted in bold

It was found that ants live together in one nest, and there were 3 times less workers *L. umbratus* than workers *L. fuliginosus* (we counted 322 workers of *L. fuliginosus* and 89 workers of *L. umbratus*). Closer to the surface, under the stone, the number of workers of *L. umbratus* was somewhat higher (43 versus 19 *L. fuliginosus* workers). It is possible that the same ratio persist in the first colony, but the majority of *L. umbratus* workers do not come to the surface, but continue to forage underground.

Colony growth rate

Among the model variants of the development of a mixed colony of two species considered by us, the first variant seems unlikely (Fig. 7A). Already in the second year, most of the *L. umbratus* workers (about 90%) will die out due to natural causes, whereas in our case, a significant number of them (27 thousand in the Kyiv colony, 22-24 thousand in the two Rivne colonies) were observed. It is unlikely that a year ago the colony of *L. umbratus* was much more numerous, in the literature there is no data on colonies of this species with the number of workers more than 100 thousand. Therefore, it can be assumed that their population is replenished with new workers of *L. umbratus* due to the fact that the queen of this species remained alive. The second option-the preservation of both queens is most likely, since we have obtained a ratio of the number of workers of both species corresponding to real measurements (Fig. 7B). Most likely, both queens can remain alive in the conditions of spatial separation in the nest. In general, this is typical for species of ants with oligogyny, when the queens are in different chambers of the nest. Variants with several queens of each species are unlikely, but we hypothetically allowed them. Thus, in the third variant, one of the two queens of *L. umbratus* remained, but the colony also included two queens of *L. fuliginosus* (Fig. 7C). If the number of *L. umbratus* remained at the level corresponding to our observations, the number of *L. fuliginosus* was 2 times larger than the real one. Therefore, we consider this option as extremely unlikely. The same applies to the last, 4th variant (Fig. 7D), when there are two queens on each side. In this case, the population of *L. umbratus* will also double, which also does not correspond to our observations and makes this option even less

likely than the third one. From the simulation, therefore, the second option is most likely, with the preservation of one queen of each species as part of a mixed colony.

It is interesting to note that in each of the three colonies surveyed, the population ratio is approximately the same - the population of *L. fuliginosus* is 2-3 times larger than the population of *L. umbratus*, which also confirms the correctness of our mathematical model. In addition, this may be a confirmation that the population of *L. fuliginosus* is growing faster than the population of *L. umbratus*, and in all cases in approximately the same proportion. The accuracy of the model is also confirmed by the results of excavation of the nests.

According to the literature, the rate of colony growth can vary greatly, depending on the ant species, its level of social organization, as well as the stage of the colony's life cycle (its age). So, according to the theoretical calculations of Tschinkel (1988), for the fire ant (*Solenopsis invicta* Buren, 1972), for 7 years of its existence, its colonies are able to reach the level of 230 thousand workers. Although the theoretical calculations were generally consistent with the results of natural population counts, it was found that there are seasonal variations in the fire ant population. The maximum number in the colony was recorded in December, but by the period from March to August, it had fallen by half. This was due to the production of sexual individuals, which coincided with the natural decline of old workers. By midsummer, the production of new fire ant workers exceeds the mortality rate of old workers (Tschinkel, 1998). On the other hand, colonies older than 5.5 years may also experience a decrease in size. The 14-year-old fire ant colonies were significantly smaller than the 5.5-year-old colonies (Tschinkel, 1988). Such fluctuations are primarily due to the lack of food resources when they are monopolized by a single (invasive in the USA) ant species. Our calculations of colony growth were carried out for the period in the first 6 years of its existence, and we took into account that *L. fuliginosus* usually has monogyny, while the fire ant most often has polygyny. Therefore, and taking into account the temperate climate of Kyiv and Rivne, we calculated the growth of the colony at a slower pace. The relationship between the temperature regime

and the colony growth rate is also interesting. In the fire ant, in the first two months of its existence, small colonies grew at a faster rate than large colonies. The optimal temperature regime was 24-32°C, with worker mortality rates of 16% in small colonies and 10% in large ones. At 35°C, mortality increased to 18% in small colonies and 50% in large colonies (Porter, 1988). In our model, constant optimal growth conditions were taken into account. To a large extent, this is facilitated by the fact that the nests of *L. umbratus*, *L. fuliginosus* are cardboard structures located either underground in the wood remains, or in cavities inside withering or dry trees, i.e. their microclimatic conditions are more stable compared to nests dug in the ground thickness.

The leaf-cutting ant (*Atta bisphaerica* Forel, 1908) demonstrates high colony growth rates similar to the fire ant. The accounting was carried out from 2 months to 32 months after the mating summer. There was a growth from 121 worker ants to 65.000 individuals (Farias *et al.*, 2020). Such high colony growth rates are probably more characteristic of tropical ant species, which have a longer optimum period when the colony is actively growing. As for the early growth period of the colony, for another species of leafcutter, *A. texana* (Buckley, 1860), the number of workers on the 90th day of the experiment varied widely – from 10 to 110 workers (Marti *et al.*, 2015).

The dynamics of colony growth in harvester ants (*Pogonomyrmex barbatus* (Smith, F.)) is somewhat lower than in fire ants and leaf-cutting ants (Gordon, 1992). Direct excavations of their nests allowed us to establish that in colonies with an age of 1 year there were no more than 100 workers, 2 years – from 1.6 to 2.1 thousand, 3 years - from 2.0 to 6.6 thousand, 4 years - from 7.2 to 10.7 thousand. Another ant species, with a more primitive social organization (*Leptothorax curvispinosus* (Mayr)) shows a lower growth rate. Thus, in 4 months, the colonies grew from 57 to 70-123 workers (Evans and Pierce, 1995).

The effect of the number of queens in a colony on the rate of its growth is interesting. In the invasive species *Paratrechina fulva* (Mayr), the following results were obtained in the laboratory: 1 queen (monogyny) – 62 larvae in 53 days, 3 queens - 92 larvae in 46 days (oligogyny), 6 queens – 156 in 49 days

(polygyny) (Arcila *et al.*, 2002). In our four variants of mixed colony growth models, the variants of monogyny and oligogyny are considered, but the variant of monogyny seems more likely, since it explains the obtained real data on the abundance of *L. fuliginosus* and *L. umbratus*. The classical work of Sommer and Hölldobler (1995) provides data on the growth of *L. niger* colonies with different numbers of queens. A feature of *L. niger* is the establishment of colonies by pleometrosis, but in the end only one queen remains (soon after the first generation of workers). For 60 days of development of colonies with one queen there were 4 workers, with 2-8, 6-19 workers. It is worth noting that the effect of pleometrosis on colony growth is important for ant species with an independent colony founding. For *L. fuliginosus* and *L. umbratus*, which are a superparasite and parasite species, respectively, size of the host colony that their queens fall into may be important. If the colony of the host species is large enough, theoretically, the growth of the parasite population will also be accelerated in the presence of its two queens. We have shown this effect in our model, in variants 3 and 4.

As for foragers on trails, their number depends on the size of the colony. Studies conducted on laboratory colonies of *L. niger* of different sizes showed that colonies of up to 25 workers at the peak of foraging relied on a maximum of 5 foragers. Colonies of 50-75 workers relied on 13-14 foragers, and large colonies of 75-200 workers on 20 foragers (Mailleux *et al.*, 2003). This means that the larger the colony, the more foragers there will be on its trail. According to Zakharov (2015), foragers make up 13% of the total population of the entire colony. In our case, with the size of a mixed colony of one hundred thousand individuals (27 thousand and 72 thousand), about 13 thousand foragers of both species should be present on the trail.

Known cases of mixed colonies of L. fuliginosus and L. umbratus

The one of the first mention of the observation of mixed colonies of *L. umbratus* and *L. fuliginosus* is recorded by Lodeizen (1946). On September 30, 1939, at the base of the oak tree, he observed a mixed nest of *L. umbratus-fuliginosus* with a strongly dominant *L. umbratus* population. In 1940, the

population of *L. umbratus* declined, but they helped in all tasks, including visiting colonies of aphids. A year later, on March 13, 1941, the first entrance to the nest was opened, and the ants that died during hibernation were placed outside the nest by *L. fuliginosus* workers. The colony of *L. fuliginosus* became very numerous and spread in all directions from the oak. Very few *L. umbratus* workers were seen at the entrance to the nest that did not leave the nest. On April 22, 1941, the last *L. umbratus* worker was seen running through the underground passages. The interpretation of this study is as follows. The putative queen of *L. fuliginosus* enters the colony of *L. umbratus* \pm June 5, 1939 (after the first period of the mating summer), kills the queen of *L. umbratus* and begins laying eggs \pm June 15, 1939. *L. fuliginosus* occurred on August 8, 1939 and quite a few *L. fuliginosus* were already outside the nest on September 30, 1939. The last worker of *L. umbratus* is discovered 1 year and 11 months after the alleged murder of the queen of *L. umbratus*. Two years is the normal life expectancy for a small proportion of workers (5%). Interpretation: the queen of *L. umbratus* was killed in June 1939, but the workers of *L. fuliginosus* were in no hurry to kill the workers of *L. umbratus* ahead of time. They more or less allowed the *L. umbratus* population to become extinct according to their natural lifespan.

Thus, the total coexistence of the two species, according to Lodeizen (1946), lasted almost three years, two of which occurred in a comparable number of workers of *L. umbratus*. According to our calculations, it takes at least 5 years for both species to reach the population level of 27,000 *L. umbratus* workers and 72,000 *L. fuliginosus* workers. Even if we observed the same colonies in Kyiv and Rivne as described by Lodeizen (1946), in Moldova a mixed population was observed by one of the co-authors of this study (VS) for 5 years, i.e. during this time the population of *L. umbratus* must have been completely extinct, since at least 4 generations of workers were replaced. The possibility of preserving the queen of *L. umbratus* seems to us to be her spatial isolation from the queen of *L. fuliginosus* within the nest. Another option is if there were several queens of *L. umbratus* and one of them remained. This is possible if the colony of *L. umbratus*, during the penetration

of the queen of *L. fuliginosus*, was large enough and the second queen (or even the only one) of *L. umbratus* was left undetected by the queen of *L. fuliginosus*. We do not claim that such mixed colonies can co-exist for a long time, but we do admit that their existence is possible. Perhaps at some point the workers of *L. fuliginosus* will kill the queen of *L. umbratus*. The question of the existence of mixed colonies will help clarify future studies of these colonies in nature or their creation in the laboratory.

Our observations differed from those made by Lodeizen (1946). Unlike his data, no aphid visits were recorded – all *L. umbratus* workers went down the oak trunk "empty", while 90% of *L. fuliginosus* workers had their bellies swollen from the fall. On the other hand, we did not observe trophallaxis between the workers of both species, but they made antenna contact equally often. The very fact of the presence of *L. umbratus* workers on the surface already indicates a complete change in their lifestyle – from underground to active visits to trees.

The colony we observed was not a collection of two contiguous nests. This is evidenced by the presence of workers of both species, coming out of the same passages to the surface in the Kyiv colony. In addition, in the colony observed in Moldova, two species of workers moved through the underground passage. Another feature of the colony from Moldova was that only *L. fuliginosus* workers came to the forage tree. Therefore, it can be assumed that the workers of *L. umbratus* do not always go outside. We assume that while there are more *L. umbratus* workers in the mixed colony, *L. umbratus* workers are more likely to be underground. *L. fuliginosus* workers also can spend most of their time in underground passages, but when the ratio changes in favor of *L. fuliginosus*; *L. umbratus* workers can massively come to the surface. We observed hundreds of colonies of *L. fuliginosus*, of different sizes. However, no *L. umbratus* workers were ever seen on their trails. This indicates the uniqueness of the possibility of the existence of mixed colonies of two species.

In the usual situation, after the first appearance of *L. fuliginosus* workers, the workers of *L. umbratus* are killed, and this is the end of the coexistence of the two species. But it cannot be excluded that in some

situations, the killing of the *L. umbratus* worker population does not occur, and they can coexist side by side for several years. It is also impossible to exclude the presence of real mixed colonies with living queens of both species, but such cases are rare, only if the queen of *L. umbratus* remains alive for some reason.

The possibility of ants of different species visiting the same trail: Parabiosis

Parabiosis is the coexistence of two species of ants within the same nest. At the same time, both species use the same trails. Parabiosis was studied in detail in the works of Menzel *et al.* (2008), between the species *Crematogaster modiglianii* Emery, 1900 and *Camponotus rufifemur* Emery, 1900. It was found that the cuticle of ants of both species was covered with steroids, which were similar in structure to each other in ants of the same nest, but different between their different colonies. The hydrocarbon profile of both species was very different, with *Cr. modiglianii* workers tolerating *Ca. rufifemur* workers from their own colony, but attacking workers from other colonies. The authors suggest that there is a mutual transfer of the substance between *Cr. modiglianii* and *Ca. rufifemur*. In this case, *Ca. rufifemur* actively or passively acquires cuticular steroids from *Cr. modiglianii*, and the latter receives at least two cuticular hydrocarbons from *Ca. rufifemur*. Thus, the transfer of cuticular steroids is possible between pairs of parabiotic species. Interestingly, both species benefited from cohabitation - *Cr. modiglianii* is more efficient in finding trophic resources, whereas *Ca. rufifemur* is more efficient in nest protection (Menzel and Bluthgen, 2010). Slightly different results were obtained for another pair of species – *Cr. schmidtii*, *Ca. lateralis* (Stukalyuk and Radchenko, 2011). *Cr. schmidtii* workers invariably attacked even *Ca. lateralis* workers from "their" nest. The fundamental difference here is that both species did not live in the same nest, but in two independent colonies located not far from each other. Based on this, it can be assumed that the exchange of cuticular compounds is possible in the case when ant species live in mixed colonies. This case also includes the four colonies of *L. fuliginosus* and *L. umbratus* that we examined. Their co-existence is possible for a few years at least. In addition, there is no mutual benefit here – one of the

species parasitizes the other, and the preservation of the queen of *L. umbratus* is a hypothetical accident. Perhaps the long-term exchange of odors between these species contributed to a decrease in aggressiveness, up to the appearance of tolerance between them and the possibility of switching to a full-fledged parabiosis instead of the classic case of superparasitism. But this statement needs to be verified. It is worth considering that the profiles of cuticular hydrocarbons in *L. fuliginosus* and *L. umbratus* differ greatly, according to Holman *et al.* (2013). On the other hand, in *L. fuliginosus*, the profiles of cuticular carbohydrate channels are similar in workers from the same colony and differ between different colonies. The profiles of all body parts were similar, except for the chest-abdomen, head, and antennae (Toshiharu and Ryohei, 2002). Therefore, the possible mixing of hydrocarbon profiles between the two species can take place exclusively in the conditions of residence of workers of the two species for at least several years.

Pheromones of the genus Lasius

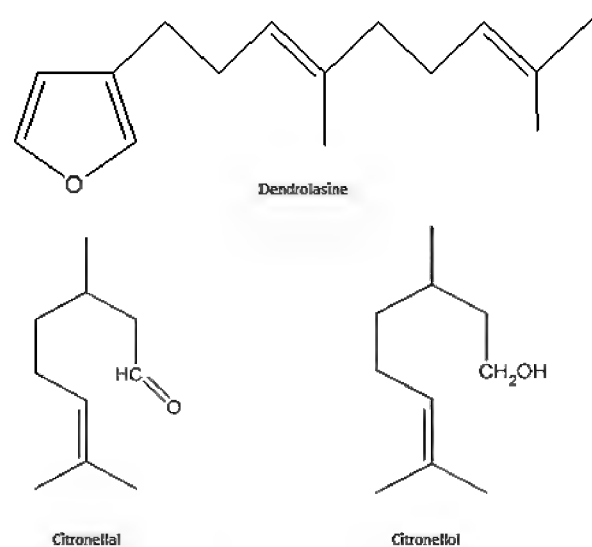
The main secretion of the mandibular glands of *L. fuliginosus* is dendrolasine (up to 86.4%, according to Bernardi *et al.* (1967), Tab. 3), in *L. umbratus* it is citronellol (85%, an alarm pheromone) and citronellal (3.4%, a protective agent, according to Blum *et al.*, 1968). These substances act as anxiety pheromones. For *L. fuliginosus*, the main abdominal extracts were: n-undecane (18.9%), n-tridecane (12.8%), dendrolasine (17%), pentadecane-2-one (35%), and heptadecane-2-one (12.4%).

Dendrolasine is a terpene with furan ring belongs to the sesquiterpenes.

Citronellal (3,7-dimethyl-6-octenal) is an aldehyde, a derivative of terpenoids (with the smell of lemon).

Citronellol (3,7-dimethyl-6-octen-1-ol) is an alcohol derivative of terpenoids (with a rose smell).

Dendrolasine and citronellol belong to different terpenes, dendrolasine has a ketone active group, and citronellol has an alcohol group. i.e. both terpenes, but with different functional groups, in *L. fuliginosus* this is a terpene with ketone, and in *L. umbratus* terpene with an alcohol group. Citronellol and citronellal are two transitional forms of the same compound, only one is more stable and numerous.



Hendecane (undecane) - a liquid hydrocarbon with the chemical formula $\text{CH}_3(\text{CH}_2)\text{CHCH}_3$, has 159 isomers is the main component in the composition of abdominal secretions in *L. alienus*, *L. carniolicus*, *L. flavus*, whereas *L. fuliginosus* has heptadecane-2-one as the main component. Heptadecane-2-one is a ketone derivative of alkane hydrocarbons of the same series as Hendecane. The composition of abdominal secretions in *L. fuliginosus* is generally qualitatively different from the rest of the aforementioned *Lasius*. Only undecane and n-tridecane in the composition of abdominal secretions are related to *L. fuliginosus* with other *Lasius* species. Also in the composition of abdominal secretions in this species, ketone derivatives of hydrocarbons and cis-citral and trans-citral predominate.

For *L. niger*, acetates are widely represented in the composition of abdominal secretions, especially given that the main component is 1-dodecyl acetate (Tab. 3). In the composition with other acetate derivatives of hydrocarbons, 1-decyl acetate, 1-hendecyl acetate, 1-tetradecyl acetate, 1-hexadecyl acetate, 1-octadecyl acetate forms a specific composition that differs from other *Lasius* species, despite the presence of a very common Hendecane. Also, the presence of a small amount of alcohol derivatives of decane series hydrocarbons makes the composition of abdominal secretions in *L. niger* very special.

For *L. alienus*, the composition of abdominal secretions is mainly represented by long-chain hydrocarbons in the presence of ketone derivatives of these hydrocarbons and a minimum content of 1-hexadecyl acetate (Tab. 3). There is also a specific component of methyl n-hexadecanoate-the methyl ester of hexadecanoic acid, which is not found in other *Lasius* species.

For *L. carniolicus*, the composition of abdominal secretions is represented by both

the hydrocarbons hendecane, 3-methylhendecane, tridecane, and a wider content of ketone derivatives of hydrocarbons 2-tridecanone, 2-pentadecanone, 3-tetradecanone, and 3-hexadecanone of similar composition than that of *L. alienus* (Tab. 3). In addition, the composition contains Citronellal, geranylcitronellal, which makes the composition of abdominal secretions different from other *Lasius* species, but similar to the composition of the mandibular gland in *L. fuliginosus*, and the presence of a ketone and an aldehyde derivative of low - mass hydrocarbons-6-methyl-5-hepten-2-one, 2,6-dimethyl-5-hepten-1-al is not characteristic of the composition of abdominal secretions other *Lasius* species, but again characteristic of the composition of the mandibular gland in *Lasius fuliginosus*. The minimum content of solid terpene alcohol-geranylgeranial is a feature of the composition of abdominal secretions in *L. carniolicus*.

The composition of abdominal secretions in *L. flavus*, although represented by the main component of hendecane, has a large number of aldehydes 4-hydroxyoctadec-9-enolide, 1-nonanal, w-aldehyde of 4-hydroxynonanolide, 4-hydroxyoctadecanolide, which makes this composition special (Tab. 3). It also contains a specific terpene-2,3-dihydrofamesal. A common component of the composition of abdominal secretions in *L. flavus* and *L. carniolicus* is citronellal.

L. umbratus has one component in common with the composition of abdominal secretions in *L. fuliginosus*, it is tridecan-2-one, as well as the composition of mandibular gland, abdominal secretions, *L. fuliginosus* hind gut extract contains many ketone derivatives of hydrocarbons (Tab. 3). The chemical composition relationship is known to be traced between *L. fuliginosus* and *L. umbratus* only by these compounds. At the same time, dendrolasine and citronellol, citronellal is quite close (terpenes), which can cause similar behavioral reactions of both species in a mixed colony in case of danger.

An interesting study was conducted in 1967 by Hangartner. Traces of *L. fuliginosus*, as well as three other species of the genus *Lasius*: *L. niger*, *L. flavus*, and *L. emarginatus* were studied. One of the conclusions of the work was that the trace substances of *L. fuliginosus* have no dependence on either the colony or the caste.

Table 3. Trace pheromones and alarm pheromones used by ants of different species of the genus *Lasius* (literature data)

Ant species and gland	Chemical name	Reference
<i>Lasius fuliginosus</i> , trail pheromones	Hexanoic acid Heptanoic acid Octanoic acid Nonanoic acid Decanoic acid	Huwyler <i>et al.</i> (1973,1975)
Mandibular gland	Dodecanoic acid Dendrolasine 6-methyl-hept-s-en-2-one, perillen, cis-citral, trans-citral, dendrolasin, farnesal	Quilico <i>et al.</i> 1956, 1957a
Abdominal secretions (possible from Dufour's gland)	Pentadecane-2-one, dendrolasin, n-undecane, n-tridecane, heptadecane-2-one (main components), cis-citral, trans-citral, tridecane-2-one (minor components).	Bernardi <i>et al.</i> , 1967
Hindgut extract	3,4-Dihydro-8-hydroxy-3-methylisocoumarin (mellein), 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	Kern <i>et al.</i> , 1997
Alarm pheromones	Decane, n-Undecane	Dumpert, 1972
<i>Lasius umbratus</i> , mandibular gland	Citronellal - releaser of alarm Citronellol - defensive secretion n-hendecane, n-tridecan-2-one	Blum <i>et al.</i> , 1968; Quilico <i>et al.</i> , 1957b
<i>Lasius niger</i> Abdominal secretions (possible from Dufour's gland)	Hendecane, 1-dodecyl acetate (main components), tridecane, heptadecene, heptadecane, nonadecane, 1-decyl acetate, 1-hendecyl acetate, 1-tetradecyl acetate, 1-hexadecyl acetate, 1-octadecyl acetate, 1-hendecanol, 1-dodecanol, 1-hexadecanol (minor components).	Bergstrom and Lofqvist, 1970
<i>Lasius alienus</i> Abdominal secretions (possible from Dufour's gland)	Hendecane (main component), tridecane, nonadecene, heneicosane, 2-tridecanone, 2-pentadecanone, 2-nonadecanone (considerable amounts), decane, dodecane, pentadecane, heptadecene, heptadecane, 2-heptadecanone, methyl n-hexadecanoate, 1-hexadecyl acetate (minor components).	Bergstrom and Lofqvist, 1970
<i>Lasius carnolicus</i> Abdominal secretions (possible from Dufour's gland)	Hendecane (main component), 2-tridecanone, 2-pentadecanone (considerable amounts), 3-methylhendecane, tridecane, 3-tetradecanone, 3-hexadecanone (minor components)	Bergstrom and Lofqvist, 1970
Head	Citronellal, geranylcitronellal (main components), 6-methyl-5-hepten-2-one, 2,6-dimethyl-5-hepten-1-al,	

Mixed colonies of *L. umbratus* & *L. fuliginosus*: when superparasitism may develop into coexistence

	geranylgeranial (minor components).	
<i>Lasius flavus</i> Abdominal secretions (possible from Dufour's gland)	Hendecane (main component), 4-hydroxyoctadec-9-enolide, 1-nonanal, w-aldehyde of 4-hydroxynonanolide, 4-hydroxyoctadecanolide (considerable amounts), decane, tridecene, tridecane (minor components).	Bergstrom and Lofqvist, 1970
Head	Citronellal, 2,3-dihydrofamesal.	

All the studied species accumulate their trace substance in the ampoule of the rectum. *L. fuliginosus* workers can interpret all traces of *L. emarginatus*, *L. niger*, with the exception of the trace substance of *L. flavus*, but the secrets in their own rectal ampoules cannot be understood by any of the other species studied. Thus, the trace substance of *L. fuliginosus* is very species-specific. This specificity does not depend on the concentration of the test solution.

The features of the interaction of *L. fuliginosus* and *L. umbratus* within a mixed colony should not necessarily be determined by the similarity of alarm pheromones and trace substances. Włodarczyk (2012) investigated the levels of aggressiveness of ants from a mixed colony of *Formica sanguinea* and *F. polyctena* to ants of the same species from single-species colonies. It was found that *F. polyctena* workers behaved more aggressively towards ants from a mixed colony, but no such aggression was detected in *F. sanguinea*. As a result, the ants of both species clearly distinguished between conspecific and allospecific ants from the mixed colony. The author concludes that as a result of social interactions, both species exchanged cuticular hydrocarbons, which led to some adjustment of their recognizable labels. Thus, the exchange of cuticular hydrocarbons can also be one of the predictors of the occurrence of mixed colonies. More recent work by Włodarczyk & Szczepaniak (2014) confirmed the hypothesis of cuticular hydrocarbon exchange. Chemical analysis of hexane extracts from the surface of ants' bodies confirmed the interspecific exchange of alkenes and alkanes with a methyl-branched chain. It is noteworthy that *F. sanguinea*, like the two species we studied, is also a parasite ("slave owner"). It is possible that parasitic and slave-owning species have adaptations that allow them to easily interact with the workers of the host species by exchanging cuticular hydrocarbons. It is also known that in

the hierarchical structure of ant associations, some subordinate species can recognize cuticular hydrocarbons of the dominant species: avoid the area with the smell of cuticular hydrocarbons of the dominant species, while the dominant species either ignore or are attracted by the smell of cuticular hydrocarbons of the subordinate species (Binz *et al.*, 2014). The authors conclude that ants of different species use heterospecific cues to avoid or seek out competitors, and that their response depends on their rank of dominance in the local community.

Conclusion

We found 4 mixed colonies that contained almost the same ratio of workers of *L. umbratus* and *L. fuliginosus* (1: 2 or 1: 3). The results obtained corresponded to the constructed model of colony growth. Presumably, such mixed colonies can exist, provided that the queen *L. umbratus* is preserved (or isolated) after the queen *L. fuliginosus* has entered the colony. Apparently, such mixed colonies are short-lived, at least in half of the cases (colonies from Kyiv and Transnistria) their life cycle ended with the death of the entire population of both species of ants. From this we can conclude that the coexistence of such mixed colonies does not exceed a period of 5 years. The first 3-4 years, such colonies grow, then the queens of both ant species are most likely to die, and the mixed colony dies out. Workers of *L. umbratus* do not take part in transporting and hunting prey, outside of the feeding area, but simply move along trails between the central and auxiliary nests. We established the presence of *L. umbratus* workers in colony 2 from the city of Rivne (Ukraine) in three distant nests, one central and two auxiliary nests. At the same time, workers of *L. umbratus* can feed on prey. It has been established that workers of *L. umbratus* can participate in the defense of the nest, leaving the passages when the enemy approaches the nest itself, and moving along the paths when

mobilizing against the enemy. It is possible that the foraging area of a mixed colony in the first years of its existence (while the colony is growing) includes 1. aboveground part, where *L. fuliginosus* mainly forages, and 2. underground part, where *L. umbratus* forages. In the future, it is necessary to conduct an experiment with the creation of a mixed colony in laboratory conditions, with the possibility of isolating the queen *L. umbratus*. It is also necessary to test the hypothesis of possible mixing of cuticular hydrocarbons between the population of the mixed colony in order to identify the reason for the tolerance of workers *L. umbratus* and *L. fuliginosus* to each other.

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A new species of *Notanisuus* Walker (Hymenoptera: Pteromalidae) from India

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Abstract

Notanisuus malabaricus Surya and Sureshan sp. n. is described with illustrations from Kerala, India. Affinities of the new species with congeneric is discussed. A key to Oriental species of *Notanisuus* is also included.

Keywords: *Pteromalidae*, *Notanisuus*, new species, key, Oriental, India.

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Introduction

Notanisuus Walker, 1837 (Cleonyminae: Cleonymini) is a small genus comprising of 18 species worldwide with three species known in the Oriental region (Gibson, 2003; Raseena *et al.*, 2014; Noyes, 2019). Members of the genus are reported to parasitize wood boring coleopterans or hymenopterans (Bouček, 1988; Gibson, 2003). The Indian representatives, *N. versicolor* (Walker, 1837) was reported from Tamil Nadu and Bihar (Bouček *et al.*, 1979; Farooqi and Subba Rao, 1986), *N. elongatus* Raseena and Sureshan from Karnataka (Raseena *et al.*, 2014) and *N. indicus* Sureshan reported from Kerala (Sureshan, 2015). Here a new species, *N. malabaricus* sp. n. is described with illustrations, from Kerala, India based on a single female specimen. The key to species of *Notanisuus* (Sureshan, 2015) is modified to incorporate the new species.

Materials and Methods

The specimen for the present study was collected using Malaise trap from Calicut University Botanical Garden in Malappuram district of Kerala. The specimens preserved in 70% alcohol were dried, card mounted and studied under LEICA M205A stereoscopic binocular microscope and imaged using attached LEICA DFC 500 camera. Measurements were

obtained using Leica LAS (Leica Application Suite V3.80). Images taken at varying focal planes were stacked using LAS and the final illustrations were enhanced for contrast and brightness using Adobe Photoshop CS5 (Version 12.0x64) software. The study is based on a single female specimen and further attempts did not yield any additional materials due to the rare nature of this genus. The holotype of the new species is deposited in the 'National Zoological Collections' of the Zoological Survey of India, Western Ghat Regional Centre, Kozhikode, Kerala (ZSIK).

Abbreviations used: The general abbreviations of the terms are as follows: **fu_x**: funicular segment, *x* being the funicle number; **Gt_x**: Gastral terga, *x* being tergum number; **mv**: marginal vein; **OOD**: ocello-ocular distance; **pmv**: post-marginal vein; **POD**: post-ocellar distance; **smv**: sub-marginal vein; **stv**: stigmal vein.

Results

Genus *Notanisuus* Walker, 1837

Notanisuus Walker, 1837: 352. Type species *Notanisuus versicolor* Walker by monotypy. Gibson (2003) may be referred for complete list of synonymy.

***Notanisis malabaricus* Surya and Sureshan**

sp. n.

(Figs. 1–10)

[urn:lsid:zoobank.org:act:C0A147B0-1BCA-4429-B099-11555403550C](https://zoobank.org/urn:lsid:zoobank.org:act:C0A147B0-1BCA-4429-B099-11555403550C)

Type material: Holotype ♀, mounted on triangular card. INDIA: Kerala, Malappuram district, Calicut University Botanical Garden (11°8'2.04" N, 75°53'26.16" E, 19m), 05.vii. 2018, Coll. C. Binoy, ZSIK Regd. No. ZSI/WGRC/IR/INV.16529

Diagnosis: The new species closely resembles *N. elongatus* Raseena and Sureshan in having: petiole 2× as long as broad; mesoscutum punctate reticulate and mesopleura almost shiny. However, the new species differs from *N. elongatus* in having: fore wing with two brown infuscations, one below *smv* and a large oval infuscation originating below parastigma reaching *pmv* at the anterior margin (in *N. elongatus*, infuscation below *mv*, *stv* and *pmv* interrupted by broad clear hyaline band extending from the anterior to the posterior end of fore wing); hind coxa punctate reticulate, 2.5× as long as broad (in *N. elongatus*, hind coxa reticulate, 1.4× as long as broad); scutellum with dense punctations in a concentric circular pattern (in *N. elongatus*, scutellum with uniform transverse punctations); *Gt*₃ largest, *Gt*₅ smaller than *Gt*₄ (in *N. elongatus*, *Gt*₄ and *Gt*₅ largest and subequal); propodeum 2.0× as wide as long (in *N. elongatus*, propodeum 1.6× as wide as long).

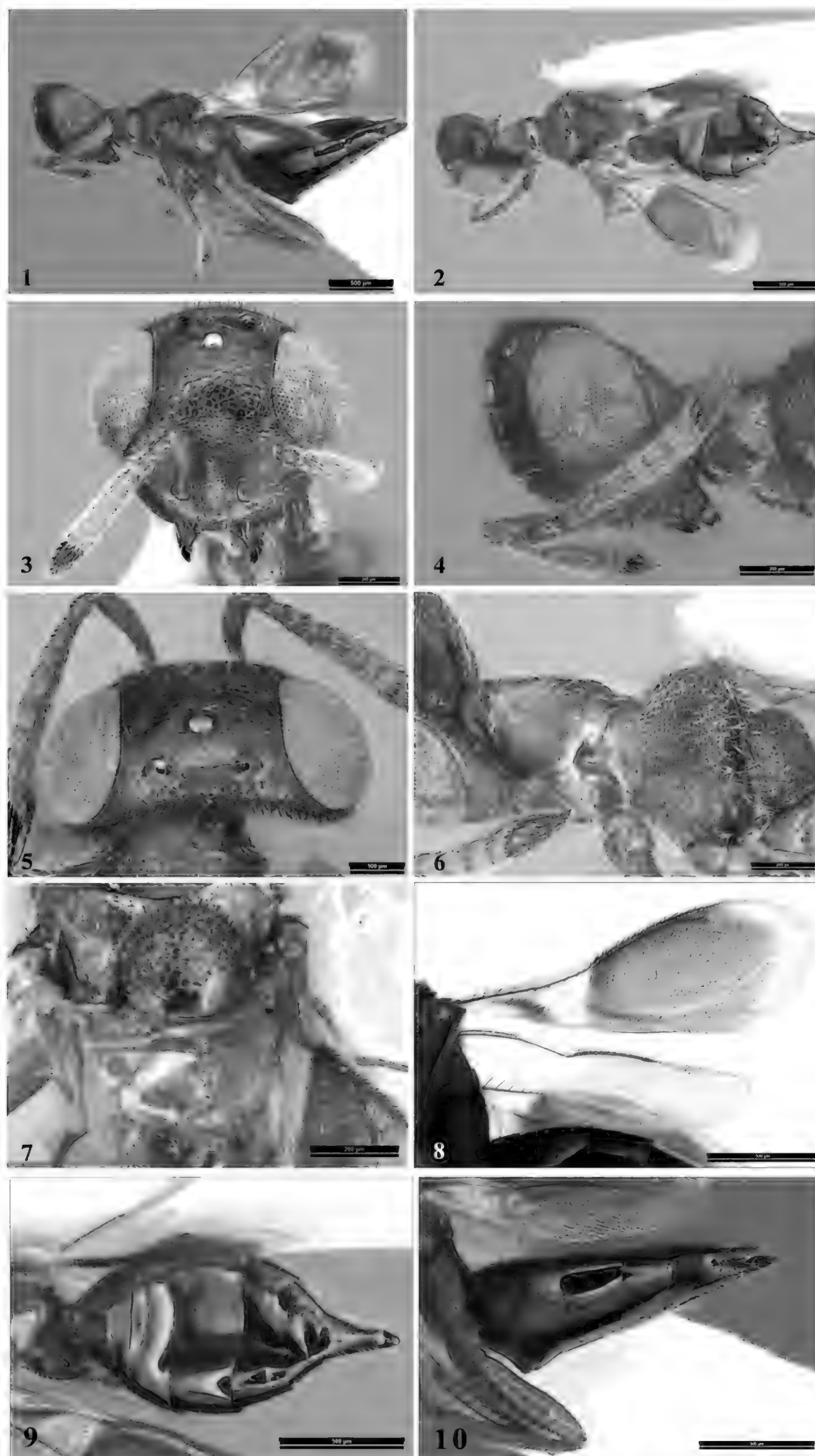
Colour description: Body honey brown except for the following: frons darker dorso-medially; antenna brown, clava black; eyes greyish; area beyond vertex and post ocellus with metallic greenish golden refringence; pronotal collar with bluish metallic refringence; mesoscutum honey brown with posterior margin extending outward and semi circularly emarginated having metallic blue refringence, slight violaceous refringence on posterior medial surface; scutellum and axillae honey brown with dark violaceous golden greenish refringence; propodeum honey brown; petiole with slight violaceous metallic refringence anteriorly; metapleura and

mesopleura posteriorly with metallic golden greenish refringence; hind coxa with metallic golden greenish and slight reddish refringence; hind tibia dorsally darker with bluish refringence; fore wing reflective, two brown infuscations, one below *smv* and a large oval infuscation from parastigma to *pmv* at the anterior margin; gaster brown, *Gt*₁ honey brown on basal half, remainder with bluish violet metallic refringence and greenish golden tint at posterior margin dorsally, *Gt*₄ and *Gt*₅ bluish with stronger bluish violet metallic refringence laterally and dorsally, remainder of metasomal terga deep brown with violaceous refringence laterally, *Gt*₆ with reddish violet refringence.

Body length: 2.45 mm; length of fore wing: 1.38 mm.

Description: Head: Quadrate in dorsal view; clypeus smooth, shiny; mandibles bidentate; head in dorsal view, 1.4× as broad as long (Fig. 5); POD 2.5× OOD; face with long white pubescence, upper half of face engraved reticulate, lower half with punctate reticulations; malar groove distinct; gena moderately engraved reticulate; eye height 1.2× width in profile, eyes minutely setose (Fig. 4); engraved reticulations strong and regular on vertex (Fig. 5); frons reticulate; occipital area moderately reticulate; antennae inserted distinctly below the lower margin of eyes, inter antennal area raised, antenna with single transverse anellus, seven funicular segments, clava three segmented; pedicel 1.5× as long as wide; scape slightly swollen towards the tip, pedicel plus flagellum 1.6× head width, pedicel 1.5× as long as broad, anellus 0.3× *fu*₁, 0.68× as long as broad, distinctly shorter than *fu*₁, *fu*₂ almost equal to *fu*₁, *fu*₃ longer than *fu*₂, *fu*₃ and *fu*₄ almost equal; club as long as three preceding segments combined (Fig. 3).

Mesosoma: Pronotum almost as long as broad, pronotal collar with distinct punctate reticulations anteriorly, posteriorly smooth and shiny, setae present laterally and near posterior margin (Fig. 6); mesoscutum 1.73× as broad as long, 0.6× as long as scutellum medially, mesoscutum punctate reticulate dorsally; metapleura anteriorly shiny and ventrally punctate; notauli incomplete; scutellum convex,



Figures 1-10. *Notanisuus malabaricus* Surya and Sureshan **sp. n.** Holotype ♀: **1.** Habitus, lateral view; **2.** Habitus, dorsal view; **3.** Head, frontal view; **4.** Head, lateral view; **5.** Head, dorsal view; **6.** Pronotum & mesoscutum, dorsal view; **7.** Scutellum & propodeum, dorsal view; **8.** Fore wing; **9.** Metasoma, dorsal view; **10.** Metasoma, lateral view.

little longer than broad, circularly punctate reticulate (Fig. 7); axillae moderately reticulate with lower margin having long white setae; dorsellum broad and shiny; propodeum 2.0× as wide as long, median carina present (Fig. 7); spiracles bean shaped and large, separated by own diameter from the hind margin of metanotum, callus shiny; mesopleura anteriorly punctate reticulate, rest shiny; legs slender, hind coxa 2.5× long as broad, densely punctate dorsally, femur reticulate (Fig. 10); fore wing 2.63× as long as broad, uncus distinct, basal half partly bare, costal cell with a row of hairs in the middle, speculum present, very small marginal fringe, 10 stiff setae present till *smv* (Fig. 8); relative lengths of *smv*, *mv*, *pmv* and *stv* in the ratio 7:4:2:1.

Metasoma: Metasoma distinctly petiolate, petiole 2× as long as broad in dorsal view, shiny with a pair of lateral carina (Fig. 9); metasoma 1.05× as long as combined lengths of head and mesosoma; Gt₂ to Gt₅ with fine reticulations anteriorly, Gt₆ completely reticulate, reticulations on epipygium distinct, posterior margins of all terga straight, Gt₁ to Gt₄ with 2–3 fine setae, Gt₅ with 4–5 setae dorso-laterally, Gt₆ and epipygium with dense brush of short setae dorso-laterally, Gt₂ smaller than Gt₁, Gt₃ largest, Gt₅ smaller than Gt₄; ovipositor and ovipositor sheath slightly exerted.

Male: Unknown.

Etymology: The species is named after the type locality (Malabar region of Kerala, India).

Distribution: India: Kerala.

Host: Unknown.

Key to Oriental species of *Notanisus* Walker (females only)

(Modified from Sureshan, 2015)

1. Fore wing with hyaline band between the fuscous patches equally wide throughout; face with uniform sculpture; antennae with anelli transverse; petiole 1.5× as long as broad.....***N. versicolor* Walker**
- Fore wing with hyaline band between the fuscous patches not equally wide, constricted towards bottom, one broad below *stv*, one narrow below parastigma; face with

differential sculpture, lower part up to little above middle distinctly reticulate punctate rest of the area finely reticulate; antennae with anelli quadrate or longer than wide; petiole shorter 1.1× long as broad.....

.....***N. indicus* Sureshan**

2. Fore wing with fuscous patches unequal, one below *mv* very long covering most of the wing, the second one below middle of *smv*, very small, infuscation below *mv*, *stv* and *pmv* interrupted by broad clear hyaline band extending from the anterior to the posterior end of fore wing; hind coxa 1.4× as long as broad; propodeum 1.6× as long as broad.....

.....***N. elongatus* Raseena and Sureshan**

- Fore wing with fuscous patches different, with two brown infuscations, one below *smv* and a large oval infuscation originating below parastigma reaching up to *pmv* at the anterior margin; hind coxa punctate reticulate with 2.5× as long as broad; propodeum 2× as long as broad.....
-***N. malabaricus* Surya and Sureshan sp. n.**

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Following the tales: The Jonah's *Ricinus* biblical worms, *Olepa schleini* in Israel a local relict or an invasive alien species

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Abstract

The members of the moth genus *Olepa* were described from the South and South Asian countries (Indomalayan realm). In 2005, a new species of moth *O. schleini* Wit, Müller, Kravchenko, Miller, Hausmann & Speidel, 2005 was described from Israel extending the geographical range of the genus to the Palearctic realm considering the species as a Paleo-Tropical relic. In 2006, this moth species was assumed to be Jonah's worm mentioned in Jonah's book 2500 years ago with the proposal of 'Critically Endangered' status. In 2012, the above status and rediscovery of 'Jonah's worm' were put for debate, as the host plant castor is not native to Israel. In early 2020, the same species and its subspecies were reported from peninsular India with genetic data indicating the possible introduction of the species from India to Israel. The recent three new species descriptions of *Olepa* moth with phylogenetic data from peninsular India supports the studies of 2012 and ascertains the possible introduction of the species from India to Israel. Discussions are made on the agricultural history of Israel and the reason for such disjunct distribution of the species *O. schleini* from Israel and India.

Keywords: *Olepa schleini*, Israel, India, castor, biogeography, invasive alien species.

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Introduction

The species of the genus *Olepa* Watson, 1980 are reported from South and South-East Asia (Walker, 2007). In 2005, *Olepa schleini* was described from the Mediterranean coastal plain of Israel by Witt *et al.* (2005). As per Muller *et al.* (2006), adult of *O. schleini* was first collected from Nahal Shiqma (southern coastal plain of Israel) during August, 1999 and the distribution was restricted to a very small area of six rivers (= Nahal) in the central and southern coastal plain of Israel (Taninim, Alexander, Ayalon and its tributary Yarqon, Shiqma and Gerar a tributary of Besor). The larvae were first collected on the Castor species *Ricinus communis* Linnaeus, 1753 (Euphorbiaceae) from Gerar in 2001 (Muller *et al.*, 2006). Muller *et al.* (2006), declared it as an endemic to the coastal plain of Israel and a Paleo-Tropical relic. They went on to declare it as 'Critically Endangered' by applying the IUCN criterion with limited sampling in Israel. As rightly questioned by Rittner *et al.* (2012) about its origin in Indian subcontinent, the present

paper aims to put forward the logic and scientific studies occurred here in India.

Reference of *O. schleini* as Biblical worm of Jonah's book

Interpretation studies by Hausmann & Müller (2006) on the tiger moth species of *O. schleini* from Israel claimed it as 'Biblical worms' from the biblical book on Jonah, and assumed it as rediscovery of the Jonah's worm mentioned in the book 2500 year ago. As per the mythological story, Jonah was a prophet not believing in the divine mercy. God wanted to teach Jonah, a lesson during 787-747 BC in Nineveh of today's Iraq. Jonah learned obedience when God gave him an object a "kikayon" and a "worm". The perfect translation for Kikayon is not available as some authors hypothesizes it as a Castor plant (Mandelkern, 1937; Simon, 1994); some says it as *Lagenaria siceraria* (Cucurbitaceae) (Amar, 2012), and in English it is translated as 'gourd', and sometimes 'vine' (Hausmann & Müller, 2006).

The origin of Castor plant (*Ricinus communis* Linnaeus, 1753)

Castor plant belongs to the Euphorbiaceae family and has four centers of origin namely, (i) Ethiopian-East African region, (ii) Northwest and Southwest Asia and Arabian Peninsula, (iii) Subcontinents of India and (iv) China (Anjani, 2012). Further, she added that Ethiopian-East African region is the most appropriate site of origin due to high diversity in Ethiopia (Moshkin, 1986; Carter and Smith, 1987; Anjani, 2012). As per Watt (1892), it is native to India based on the references of medicinal uses of the plant in Sanskrit literature and evidences at the foothills of Himalayas. ICAR-IIOR (2020), suggested four centres of origin: West Asia, South West Asia, India and China.

In 2013, Israel government published a list of “Israel's Least Wanted Alien Ornamental Plant Species” and castor was in that list as Invasive (Invasion stage) (Dror, 2013) plant. Castor entered Israel through Africa in ancient times (Rittner *et al.*, 2012). India shares largest area (11.48 lakh ha.) and highest productivity (1666 kg/ha) of castor in the world (Anonymous, 2020). India, Brazil, USSR, China and Thailand are the major castor producing countries and the major importing countries are the USA, the USSR, the EEC and Japan (Sujatha *et al.*, 2011).

Muller *et al.* (2006) reported the larvae of *O. schleini* feeding on castor plant and is the first known host plant. Witt *et al.* (2005) termed it as monophagous pest on castor. Further, Rittner *et al.* (2012) added nine new host plants (*Cussonia paniculata*; *Ipomoea batatas*; *Brassica oleracea*; *Epipremnum pinnatum*; *Strobilanthes anisophyllus*; *Barleria obtuse*; *Nicotiana glauca*; *Passiflora edulis*) and stated all these host plants are not native to Israel.

Record of the species *O. schleini* from Peninsular India

During our collection survey from the peninsular India, some of the collected specimens were exactly matching the morphological descriptions and male genital characters of *O. schleini* from Israel. As the Oriental and Palearctic regions shared different zoogeographies, the specimens were put for DNA barcode studies. From the DNA barcode studies, the specimens were confirmed to be *O. schleini* from India, making it a new

record for the country (Kalawate *et al.*, 2020a). In the same study, a subspecies i.e., *O. schleini chandrai* Kalawate, 2020 was described based on morphological, genitalia and barcode studies. It has contrasting morphological difference with *O. schleini* from Israel and shallow genetic diversity on the mt COI DNA.

Materials and Methods

The present study was taken up to prove the logic behind the origin of the *O. schleini*. The map of the extant species recorded from the South and South-East Asia is prepared using open free QGIS software. The phylogenetic tree was adopted and modified from Kalawate (2020b). To understand better the occurrence of the species from the genus and their distribution a checklist has been prepared and presented in Table 1 with their host plant. In the forgoing pages we will see the concurrence of the Palearctic species in the peninsular India.

Results and Discussion

From the map and the table 1 it is clear that the origin of the species of the genus *Olepa* are from the South and South East Asian countries with the only exception of *O. schleini*.

Biogeography, taxonomic studies and inferences on the range of distribution

In Israel, *O. schleini* was collected under a long-term project started in 1986 to study insect fauna (focusing mainly on Lepidoptera) in a collaborative effort with the Nature and Parks Authority of Israel, the Tel Aviv University, the Hebrew University Jerusalem, and the Museum Witt, Munich, Germany (Muller *et al.*, 2005). Israel is an agriculturally developed country and supposed to be a world-leader in agricultural technologies including drip irrigation and squeezing water drops from air (Anonymous, 2021a). Israel and Indian agricultural scientist are sharing lot of technical knowledge. India has made Israel as a strategic partner in the field of Agriculture under the Indo-Israel Agricultural Project (IIAP) (Anonymous, 2021b). This technical know-how is being shared by Israel to other countries and there are chances that during these technology transfers and sharing of biological resources the members of the species *O. schleini* might have introduced to Israel unknowingly. This has been clearly established in the studies of

Kalawate *et al.* (2020a), where different population of *O. schleini* in India and Israel having same morphological features and genetic homogeneity (mt COI gene) were studied. Supporting the above statements Kalawate *et al.* (2020b) have described three new species and a sub species from peninsular India. The phylogenetic tree based on the mt COI gene fragment (599bp) suggests *O. toulgoeti* reported from Nilgiri hills of the Western Ghats as a sister species to *O. schleini* described from Israel. From Fig.1 and Table 1 it is clear that the centre of origin of this group is South and South-East Asia mainly from India, Sri-Lanka and Thailand.

As per the earlier reports, if *O. schleini* is endemic and paleo-tropical relic of Israel, then the host plant should also be a local species. Nevertheless, in this case, castor is not native to Israel and the biblical event in Jonah's book was believed to have taken place some 2500 years ago. Agricultural developmental activities in Israel are recent compared to biblical event of Jonah's book. In such an agriculturally developed country, how this moth was undiscovered these many years is a brilliant argument put forth by Rittner *et al.* (2012). Hausmann & Müller (2006) stated that only one lepidoptera i.e. *Dysgonia algira* (Linnaeus, 1767) was recorded as a pest of castor in the literature until the recent discovery of *O. schleini*, a leaf eater in Israel.

On the contrary, *Dialeurodes citri* (Ashmead, 1885) (Hausmann & Müller, 2006), *Phycita diaphana* Staudinger, 1870, *Stomphastis conflua* (Meyrick, 1914), *Spodoptera exigua* (Hübner, 1808), *Dysgonia algira* (Linnaeus, 1767) were the pest reported by Rittner *et al.* (2012) in Israel for castor other than lepidopteran pest.

In India, the major pests reported on castor are: *Amsacta albistriga* (Walker, 1865), *Achaea janata* (Linnaeus, 1758); *Spodoptera litura* (Fabricius, 1775); *Conogethes (Dichocrosis) punctiferalis* (Guenée, 1854); *Empoasca flavescens* (Fabricius, 1794); *Retithrips syriacus* (Mayet, 1890); *Trialeurodes ricini* (Misra, 1924); *Liriomyza trifolii* (Burgess, 1880); *Spilosoma obliqua* (Walker, 1855) and *O. ricini* (Lepidoptera: Erebidae) (ICAR-NBAIR, 2019).

The reported pests are either foliage feeder or sap-sucker and not stem-borer (eater) as mentioned in the Jonah's book. The worm mentioned in the biblical book seems like stem

borer as it was gnawing the stem and withering of the plant occurred in a night. *O. schleini* is a leaf-eater (Rittner *et al.*, 2012). Hence, as rightly stated by Rittner *et al.* (2012), the biblical worm is not *O. schleini*.

Out of the eleven reported species of *Olepa* from the world, nine species are from India and one each from Sri Lanka and Thailand (Fig. 1) (Kalawate *et al.*, 2020b). Recently, Kalawate *et al.* (2020a, b) described three new species namely *O. ghatmatha* Kalawate, 2020; *O. suryamal* Kalawate, 2020; *O. zedesi* Kalawate, 2020 and two subspecies, *O. suryamal rekhae* Kalawate, 2020 & *O. schleini chandrai* Kalawate, 2020 including a new country record for *O. schleini*. With these additions, now the species in the genus *Olepa* stands at 14 from the world including 12 species and 2 subspecies from India.

The type locality of *O. schleini* from Israel and the place where it is reported in India are separated by a minimum aerial distance of 4,000km. There seems to be disjunctive distribution range for the species from India and Israel without having their distribution in between countries. To date no other species of *Olepa* are recorded out of India, Sri Lanka, Nepal, Northern Pakistan and Thailand except *O. schleini* (from Israel). Based on our earlier mt DNA barcode studies on *O. schleini* (from Nandurabr, India) (Kalawate *et al.*, 2020a) and reports of new species based on mt DNA barcode studies from peninsular India (Kalawate *et al.*, 2020b) we are of the opinion that origin and centre of diversity of this Tiger moth could be South or South east Asia.

Conclusion

Based on our mt DNA barcode studies and field sampling of the *Olepa* moths in India we defer the Muller *et al.* (2006) consideration of *O. schleini* endemic to the coastal plain of Israel and we warrant further molecular studies to consider the species as Paleo-Tropical relic (Witt *et al.*, 2005).

We support the arguments of Rittner *et al.* (2012) regarding the feeding behavior of *O. schleini* and the the origin of castor plant which is a host plant for *O. schleini* in Israel. We consider it premature to consider *O. schleini* as 'Critically Endangered' (Muller *et al.*, 2006) without studying the distribution range of the species. The 'Biblical worms' mentioned in the Jonah's book could be a stem

borer as mentioned by Rittner *et al.* (2012) and *O. schleini* could be very recently introduced

to Israel from India through the host plants during the Israel's new agricultural reforms.

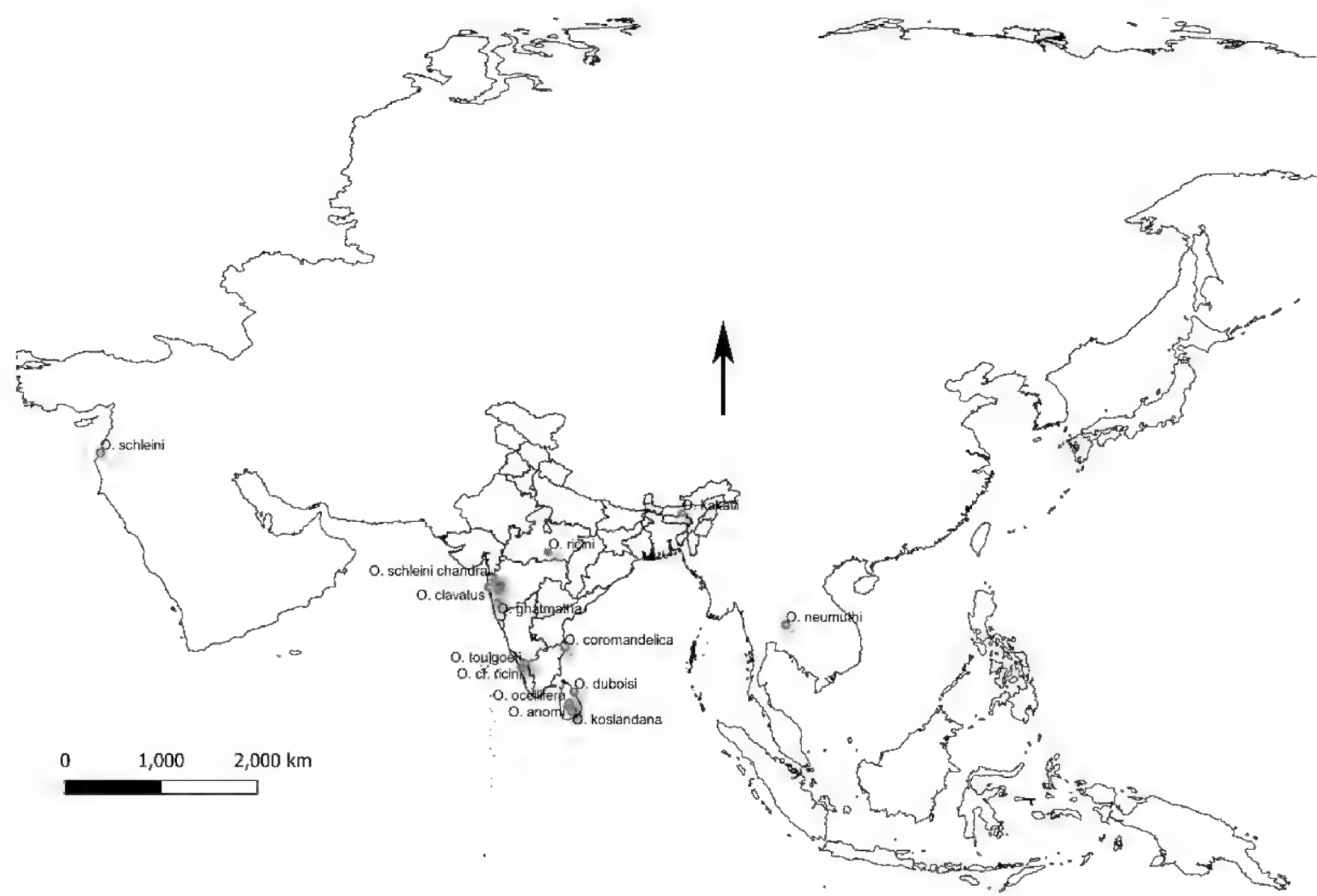


Figure 1. Map showing the type localities for the extant species of *Olepa* from South, South East Asia and Israel.

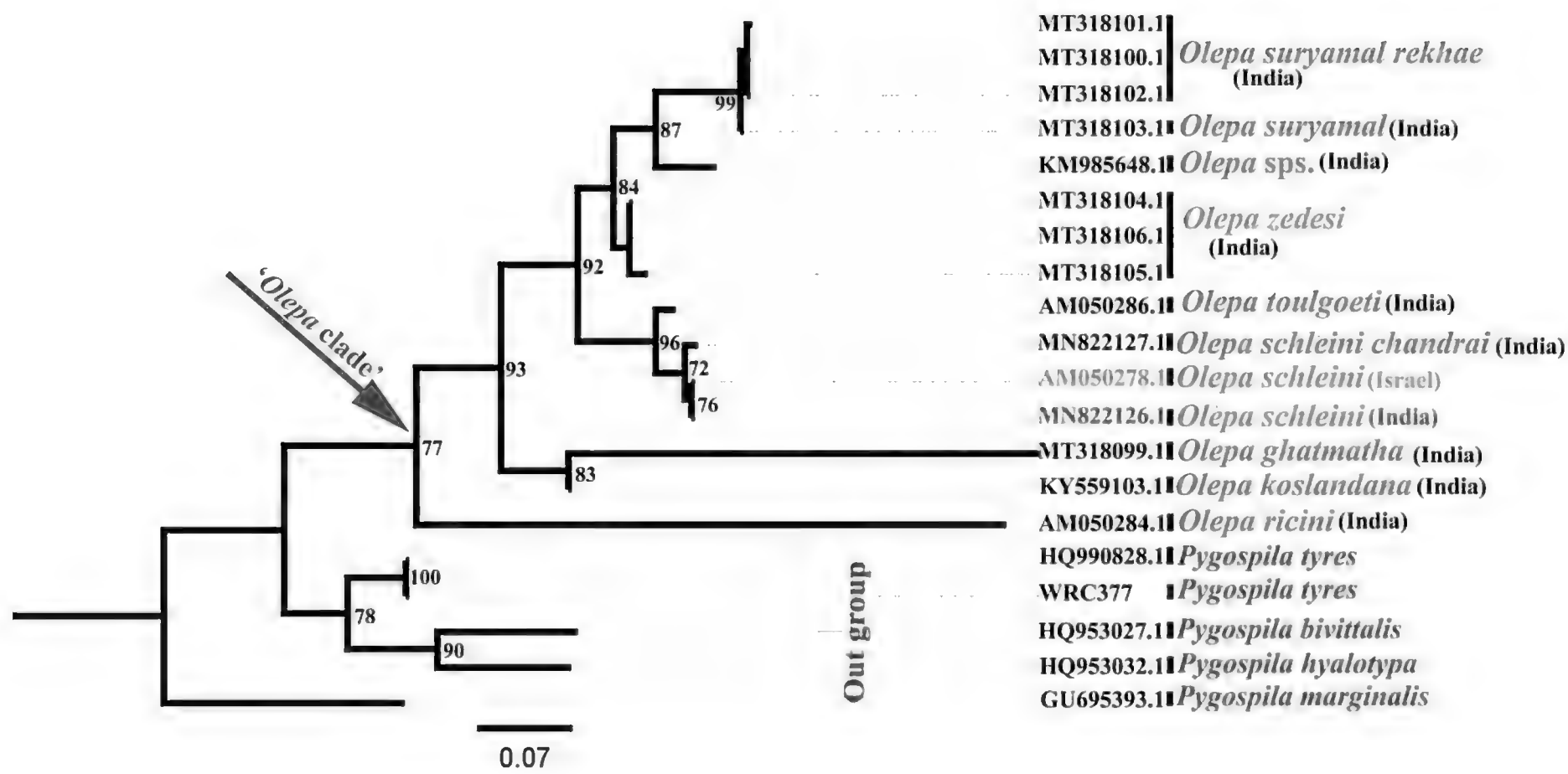


Figure 2. mt COI DNA based (599 bp) Maximum Likelihood (ML) tree for the species of *Olepa* moths (details as in Kalawate *et al.*, 2020b).

Table 1. The known host plant species of *Olepa* species with their distribution ranges

Sl. No.	Species	Known distribution ranges (Kalawate, 2020b)	Known host plant details
1	<i>O. ocellifera</i> (Walker, 1980)	Bangladesh, India (Indian Himalaya, Assam), Sri Lanka	<i>Notonia</i> (Asteraceae); <i>Fagraea</i> (Loganiaceae); <i>Dahlia</i> (Asteraceae) (Moore 1883)
2.	<i>O. duboisi</i> Orhant, 1986	India (Kerala), Sri Lanka	Pissenlit (Dandelion); Plantago (Plantain) (Plantaginaceae); <i>Cichorium</i> (endives) (Asteraceae) (Orhant, 1986)
3	<i>O. anomi</i> Orhant, 1986	Sri Lanka	Not known
4	<i>O. kakatii</i> Orhant, 2000	India (Assam)	Not known
5	<i>O. ricini</i> (Fabricius, 1775)	Bangladesh, Nepal, Northern Pakistan, Sri Lanka, Thailand throughout India,	Cotton (Malvaceae); castor (Euphorbiaceae) (Fabricius, 1775); sunflower (Asteraceae); sesame (Pedaliaceae); maize (Poaceae); ivy gourd (Cucurbitaceae); brinjal (Solanaceae); sweet potato (Convolvulaceae); banana (Musaceae) (ICAR-NBAIR 2019); polyphagous (Gaur and Kumar, 2019).
6	<i>O. schleini</i> Witt <i>et al.</i> , 2005	India (Maharashtra), Israel	Castor (Euphorbiaceae) (Witt <i>et al.</i> , 2005)
7	<i>O. neumuthi</i> Orhant, 2012	Thailand	Castor (Euphorbiaceae) (Orhant, 2012)
8	<i>O. clavatus</i> (Swinhoe, 1885)	India (South India, Maharashtra)	Not known
9	<i>O. toulgoeti</i> Orhant, 1986	Orissa, South India	Not known
10	<i>O. koslandana</i> Orhant, 1986	India (Karnataka, Coromandel, Orissa, Bihar, Jharkhand), Sri Lanka (koslanda)	Not known
11	<i>O. coromandelica</i> Dubatolov, 2011	India (Coromandel)	Not known
12	<i>O. ghatmatha</i> Kalawate, 2020	India (Maharashtra)	Not known
13	<i>O. suryamal</i> Kalawate, 2020	India (Maharashtra)	Not known
14	<i>O. zedesi</i> Kalawate, 2020	India (Maharashtra)	Not known
15	<i>O. schleini chandrai</i> Kalawate, 2020	India (Maharashtra)	Not known
16	<i>O. suryamal rekhae</i> Kalawate, 2020	India (Maharashtra)	Not known

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Species composition of mites (Acari: Trombiculidae, Dermanyssidae, Laelapidae) on pest rodents and shrews in Madurai, India

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Abstract

Trombiculid chigger mites were collected from pest rodents and shrews. This study was carried out in different habitats like urban, semi-urban, and rural sites in Madurai district, South Tamil Nadu, India during 2017-2018. A total of 3219 chigger mites comprising 12 different species under three families (Trombiculidae, Dermanyssidae and Laelapidae) and one sub order (Oribatida) were collected from 151 rodents and shrews, belonging to six different species in this study. The chigger infestation rate in these small mammals was 56.47. The following chigger mites and adult mite species were identified from the Madurai district: *Leptotrombidium deliense*, *L. keukenschrijveri*, *L. indicum*, *L. rajasthanense*, *Schoengastiella ligula*, *Microtrombicula* sp., *Neotrombicula microti*, *Trombicula hypodermata*, *Schoengastia* species (Chigger mites); *Dermanyssus gallinae*, *Laelaps echidninus*, *Liponyssoides sanguineus* and Oribatida (Adult mites). The predominant species recorded from these areas are *L. deliense* (65.94%), *L. indicum* (11.50) and *S. ligula* (15.27). Even though maximum chigger mites infestation rate was recorded in rodents and shrews from urban areas (65.5), followed by semi-urban (53.5) and rural (54.5) areas. Trap positivity rate (18.1%), rodents/shrew captures (5.42 ± 2.97) and chigger collection (118.08 ± 188.9) were observed significantly ($P < 0.05$) more in rural clusters compared with urban clusters. This study showed the predominant trombiculid mites activity on small mammals of this place which can forewarn the public health officials to understand the ground-level situation to take up necessary control measures for the prevention of mite borne diseases.

Keywords: Mites, habitats, species composition, rodents, shrews.

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Introduction

Chigger mites commonly known as chigger trombiculid mites, soil mites, grass itch mites, and scrub-itch mites, belong to the family Trombiculidae, Dermanyssidae, Laelapidae and Sarcoptidae, superfamily Trombidioidea, order Trombidiformes and Sarcoptiformes in subclass Acari of class Arachnida.

Scrub typhus is a vector-borne human disease caused by obligate intracellular bacterium *Orientia tsutsugamushi* which is transmitted by a species of the Trombiculidae family (Walker *et al.*, 1975; Chakraborty and Sarma, 2017; Elliott *et al.*, 2019; Prakash *et al.*,

2021). Larval stages of mites are ectoparasites harbored by rodents and shrews which in turn act as the reservoirs for this bacterium. A survey was carried out to investigate the prevalence of Trombiculid mites, the vectors for scrub typhus on pest rodents and shrews in Madurai district, Tamil Nadu at three different habitats such as urban, semi-urban, and rural sites to know the species composition and distribution pattern. The study sites were selected based on the areas with a nuisance of pest rodents as reported by the residents. This study helped to delineate the mite-infested areas to initiate the vector control

strategy.

Materials and Methods

Study sites

Madurai district is located in south of Tamil Nadu State of India, lies between 9°33'30''N to 10°18'50''N Latitude, 77°00'00'' E to 78°30'45''E Longitude and has an area extent of 3741.73 sq. km. (District Statistical Handbook, 2018). Nine study sites were selected, grouped into urban, semi-urban, and rural habitats with three sites each: B.B.Kulam, Tirumangalam, Usilampatti (urban habitats), Peraiyur, Keelaiyur, Sholavandan (semi-urban habitats), Vadapalanji, Katchaikatti, and Chatrapatti (rural habitats) (Fig. 1).

Collection of trombiculid mites from rodents/shrew:

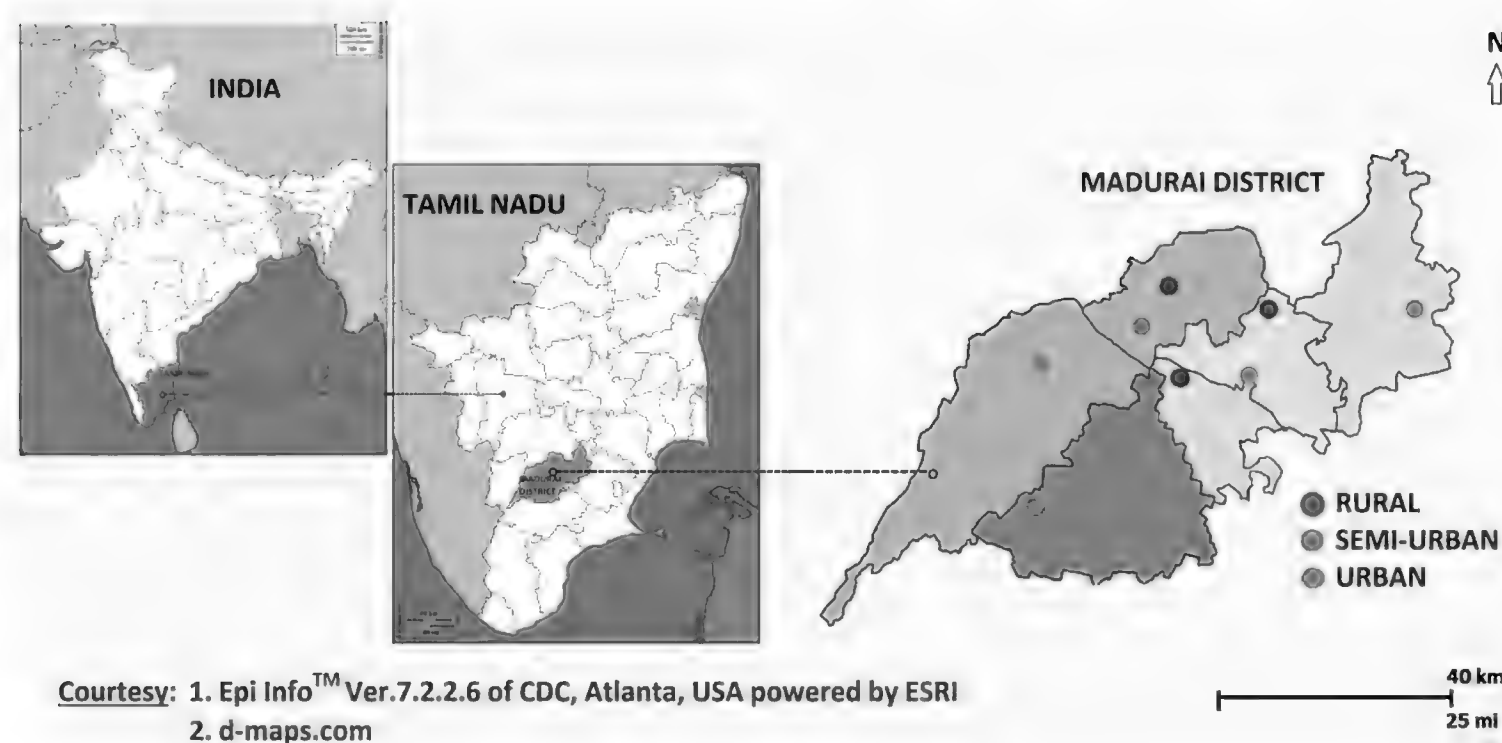


Figure 1: Map showing different study sites

Trombiculid mites' collection & identification:

Chigger mites were collected from the pest rodents and shrews. Ear pinna and femur were the major sites of collection of mites (Fig. 2). Using a fine brush (size: 2 mm) and Burette, all chigger mites were collected from the host body. The collected mites were stored in 70% ethanol. After 6 hours, the collected chigger mites were transferred to lactophenol solution (lactic acid 50 ml, phenol 25 ml, and distilled water 25 ml) for clearing the specimens. After clearing, all mite specimens were mounted on

At every site, Sherman traps (width 7.5 cm; length 18.5 cm; depth 9 cm) (Samuel *et al.*, 2020) were kept in and around residential areas at indoor and outdoor households before dusk (5-6pm) and withdrawn after dawn (6-7am) the next day. All the rodents were attracted by fried eatables smeared with coconut oil kept within the Sherman traps and captured (Samuel *et al.*, 2020). Pest rodents and shrews were identified based on taxonomic keys of Shakunthala and Tripathi (2005) and Martin *et al.* (2011). 360 Sherman traps were placed in each urban, semi-urban and rural site during this study period. All the trapped rodents were placed in separate cloth bags and brought to the laboratory. Captured rodents were anesthetized for the collection of mites (Samuel *et al.*, 2020, 2021a). Six genera of pest rodents and shrews were trapped. A variety of mites belonging to different genera and species were collected from the infected rodents.

separate slides with Hoyer's medium (Samuel *et al.*, 2021b). Slides were studied under Nikon ECLIPSE (E200) microscope and identified up to species level using keys by Stan Fernandes and Kulkarni (2003), Goff *et al.* (1982) and Nadchatram *et al.* (1974). These slides were deposited in Mosquito Museum Entomology Laboratory under the Unit of Vector-Borne and Zoonotic Disease, ICMR-Vector Control Research Centre Field Station Madurai, Tamil Nadu. This study was approved by the Institutional Animal Ethics Committee (IAEC) of ICMR-VCRC, Puducherry.



Figure 2: A. Fixing Sherman trap; B, C. Collection of chigger mites from ear pinna and hind femur

Data Analysis

Data were entered into Excel software (Microsoft Corp.) and analyzed using STATA software (version 15.0, Stata Corp. LLC). Categorical data are presented as frequencies and continuous data are summarized as mean (\pm standard deviation). Independent T-test and Chi-square test were used to compare cluster differences. For GPS-based spot mapping of the study site and distance measurements, Epi Map of Epi Info Ver. 7.2.2.6 of CDC, Atlanta, USA powered by ESRI was used. Maps of Indian states and districts were downloaded from d-map (2021).

Results

Totally 1080 traps were fixed in the nine study sites (120 traps in each site) during one year and each cluster shared 360 traps. Five different pest rodent species and one shrew species were trapped in all urban, semi-urban and rural clusters, in the Madurai district. A total of 35(9.7%), 51(14.2%), and 65(18.1%) rodents and shrews were trapped at urban, semi-urban, and rural sites respectively (Fig. 3). Among the trapped rodents and shrews, 65.47 ± 107.79 , 84.67 ± 135.12 and 118.08 ± 188.9 chigger mites were collected at urban, semi-urban, and rural sites respectively (Fig. 4). A significantly more ($P < 0.05$) number of positive traps and number of chiggers were collected in rural and semi-urban clusters compared with urban clusters (Table 1).

A total of 786 mites were collected from urban areas, of which 413 belonged to six different species, 648 out of 1016 mites from semi-urban areas belonged to seven different species and 1035 out of 1417 mites collected from the rural area represented eleven different species (Table 2). A high proportion of chiggers were collected from the *Rattus rattus* (79.1%).

In total 3219 chigger mites and 151 rodents and shrews were captured from urban, semi-urban, and rural habitats. A total of 2,096 individual trombiculid chigger mites belonging to nine species were collected from the Trombiculidae family. In our present study, *R. rattus* and *Suncus murinus* were more vulnerable than the rest of the rodent species. 1707 and 1168 chigger mites were collected from 32 *R. rattus* and 17 *S. murinus* respectively. From the rest of the rodent species *R. norvegicus* (190), *Tatera indica* (142), and *Bandicota bengalensis* (154) mites were collected. *Leptotrombidium deliense* was the predominant species (76%) followed by *S. ligula* (15%), *L. indicum* (5%), *L. rajasthanense* (2%), and *Schoengastia* sp. (2%) in urban sites. *L. deliense* was also the predominant species (67%) following *S. ligula* (17%), *L. indicum* (11%), *Trombicula hypodermata* (2%) and *L. rajasthanense* (1%) in semi-urban sites. *Leptotrombidium deliense* was the predominant species (62%) following *S. ligula* (14%), *L. indicum* (14.0%), *T. hypodermata* (2%), *L. rajasthanense* (2%), and *Microtrombicula* sp. (1%) in rural sites. *L.*

deliense was the most prevalent on all hosts (Table 2).

Table 1. Comparison of rodent trap positivity, chigger collection and infection rate between the clusters in Madurai district, Tamil Nadu 2017-2018

Cluster type	Trap Fixed (N)	Trap Positivity rate % (n)	Rodent/ Shrews catches (Mean \pm SD)	Rodent/ Shrews infection Rate % (n)	Chigger infection rate (n)	No. of Chigger collected (Mean \pm SD)	Chigger Index
Urban	360	9.7 (35)	2.92 \pm 1.31	34.3 (12)	65.5 (786)	65.47 \pm 107.79	22.46
Semi-Urban	360	14.2 (51) *	4.25 \pm 2.30	37.3 (19)	53.5 (1016)	84.67 \pm 135.12 *	19.92
Rural	360	18.1 (65) *	5.42 \pm 2.97 *	40.0 (26)	54.5 (1417)	118.08 \pm 188.9 *	21.80
* Significantly differed from Urban (P <0.05)							

Table 2. Chigger/Adult mite species collected from rodents/shrews captured at South Tamil Nadu, India

S.No	Mites species	Urban	%	Semi Urban	%	Rural	%
1	<i>Leptotrombidium deliense</i> (Walch,1922)	311	75.54	431	66.82	640	62.13
2	<i>L. keukenschrijveri</i> (Walch,1923)	0	0.00	11	1.70	25	2.42
3	<i>L. indicum</i> Fernandes and Kulkarni, 2003	21	5.08	71	10.96	149	14.40
4	<i>L. rajasthanense</i> Fernandes and Kulkarni, 2003	9	2.18	9	1.39	24	2.32
5	<i>Schoengastiella ligula</i> Radford, 1946	62	15.01	109	16.82	149	14.40
6	<i>Microtrombicula</i> sp. Ewing,1950	0	0.00	0	0.00	14	1.35
7	<i>Neotrombicula microti</i> (Ewing,1928)	0	0.00	0	0.00	7	0.68
8	<i>Trombicula hypodermata</i> Nadchatram and Traub,1966	0	0.00	15	2.31	23	2.22
9	<i>Schoengastia</i> sp. Vercammen-Grandjean 1960	9	2.18	0	0.00	0	0.00
10	<i>Dermanyssus gallinae</i> (De Geer, 1778)*	0	0.00	0	0.00	2	0.19
11	<i>Laelaps echidninus</i> (Koch,1836)*	0	0.00	0	0.00	1	0.10
12	<i>Liponyssoides sanguineus</i> Lavine and Large (1984)*	0	0.00	2	0.31	1	0.10
13	Sub order: Oribatida; Duges, 1833*	1	0.24	0	0.00	0	0.00
Total		413		648		1035	

*Adult mites

Species composition of mites on pest rodents and shrews in Madurai, India

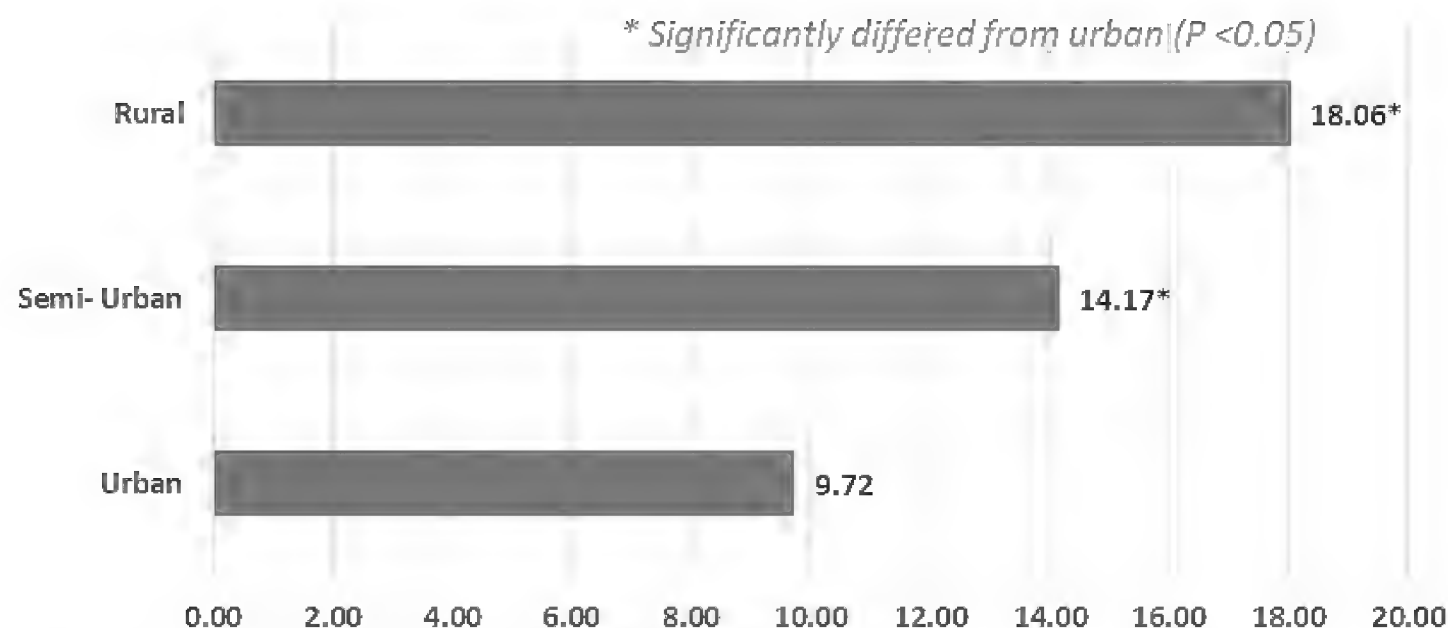


Figure 3: Details of trap positivity in different habitats

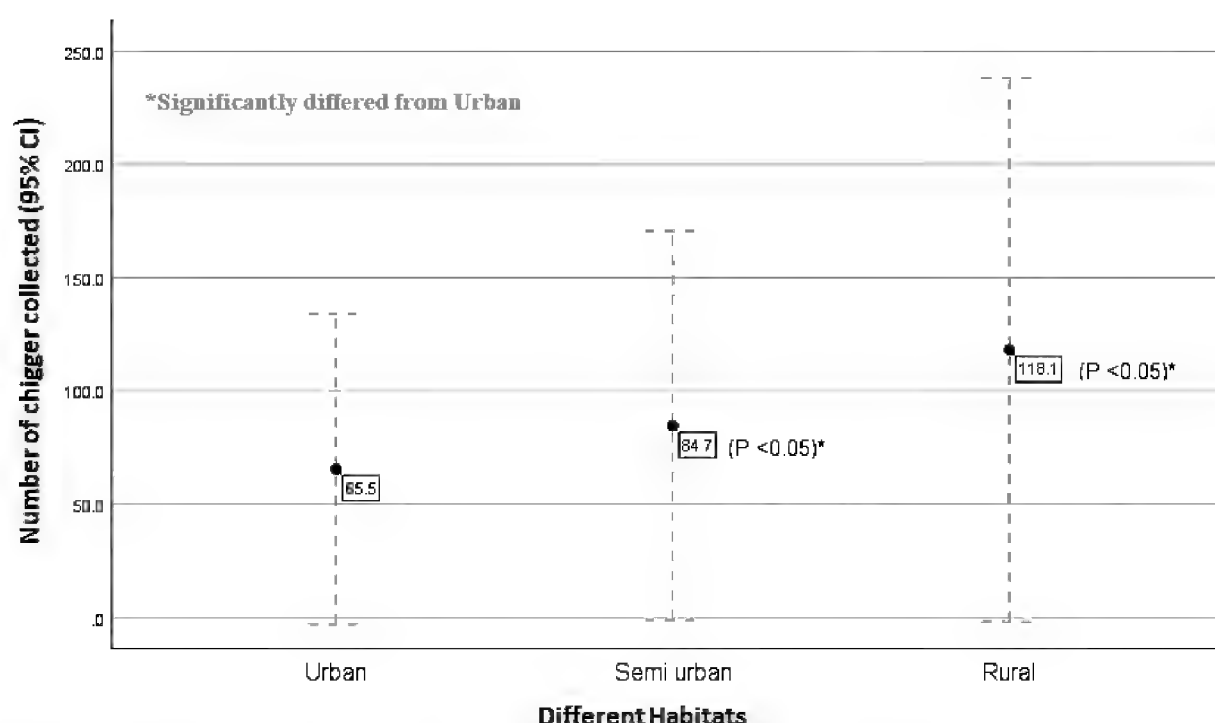


Figure 4: Trombiculid mites' collection details from different habitats

Discussion

In the worldwide distribution pattern of Acarines, approximately 12% of 50,000 species live as parasites, and less than 100 species are reported to have medical and veterinary importance (Krantz, 1978; Krantz and Walter, 2009). Many Acarines act as vectors of zoonotic diseases and live very close to the animal host. Moreover, environmental degradation led to global climate changes in many places. This led to the proliferation of the vectors and in turn transmission of rodent-borne parasitic diseases (Paramasvaran *et al.*, 2009). Many ectoparasites of rodents play a crucial role in disease transmission to humans and animals. Thus, these studies on ectoparasites have invaluable data to take up appropriate control program. Medically important chigger mites, *Ascoshengastia* sp.,

L. deliense, *L. jayawickremei*, *L. rajasthanense* and *S. ligula* are the powerful vectors of scrub typhus (Prakash *et al.*, 2021).

Fernandes *et al.* (1988) collected 11,883 chiggers belonging to 65 species in 14 genera from the Himalayan region. The collection included 5 genera, *Leptotrombidium*, *Neotrombicula*, *Microtrombicula*, *Schoengastia*, and *Schoengastiella* recorded from the present study. In 1966, *L. akamushi*, *L. deliense*, and *S. ligula* were collected from Eastern Himalayan foothills (Varma and Mahadevan, 1971). Except *L. akamushi*, the other 2 species of mites were collected in this study. A survey conducted between 1966 and 1970 in Western Himalayas, Sikkim, and hill districts of West Bengal showed 66 species of Ixodoidea, 15 species of Mesostigmatid mites, and a large number of

Trombiculid mites in addition to other hematophagous arthropods (Rao *et al.*, 1973). An outbreak investigation conducted in Kurseong, Darjeeling established *S. ligula* as the vector of scrub typhus due to its abundance (Tilak *et al.*, 2011).

Earlier studies have documented more than 3000 species of chiggers all over the world and over 400 mite species were recorded in China (Peng *et al.*, 2016). A total of 274 species of mites were collected from Yunnan Province, southwest China. Fujian Province documented 53 species of chiggers, Hubei Province recorded 41 chigger mite species and Beijing recorded 7 species of chigger mites (Peng *et al.*, 2016). In India so far 204 chigger mite species were recorded by Stan Fernandes and Kulkarni (2003). In our study conducted in Thiruvananthapuram district, Kerala, we reported 18 species of chiggers and one adult mite (Samuel *et al.*, 2020). Similarly our study carried in the scrub typhus affected areas in various districts of Tamil Nadu showed the presence of chigger mites and adult mites (Samuel *et al.*, 2017, 2021a). In the present study conducted in different habitats like urban, semi urban and rural areas, we collected 9 species of chiggers belonging to 6 genera and falling under family Trombiculidae and three adult mite species belonging to the 2 families (Dermanyssidae and Laelapidae) and one sub order (Oribatida). This study recorded seven genera, 12 species and one sub order Oribatida of mites from Madurai from six species of rodents and shrews. In Egypt, *Mus musculus* harbored fewer ectoparasites but the other species like *R. rattus frugivorus*, *R. norvegicus*, and *Meriones shawi* were vulnerable to ectoparasites (Kandy *et al.*, 2007) and in Tamil Nadu, India, *Rattus rattus*, *R. norvegicus*, *M. musculus*, and *S. murinus* were found vulnerable for ectoparasite infection (Samuel *et al.*, 2021a). This present study conducted during one full year explored the ectoparasitic mite fauna in the Madurai district of Tamil Nadu, India. We could demonstrate the rodent-associated ectoparasites present in all these study sites. *Leptotrombidium deliense*, *S. ligula* and *L. rajasthanense* collected aplenty in this present collection from Madurai were considered as the scrub typhus vectors from

India (Prakash *et al.*, 2021). Three adult *D. gallinae*, *L. sanguineus* and *L. echidninus* mite vectors of human pathogens reported worldwide were recorded from the rural collection (Azad, 1986).

A total number of the vertebrate host species play a very significant factor to influence the species composition of chigger mites from different places. From this study, 4 species of vector mites reported in India were recorded. The abundant collection of these species denotes the potential risk for the transmission of this emerging disease in these areas. Scrub typhus is the most common re-emerging Rickettsial infection in India and many other Southeast Asian countries. Monitoring of the rodent population and their trombiculid mites brings forth important data to facilitate arthropod-borne disease control strategies by the public health authorities.

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Description of a new species of *Antrocephalus* Kirby (Hymenoptera: Chalcididae) from Kaippad paddy field of Kerala, India

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Abstract

A new species of *Antrocephalus* is newly described with illustrations from Kaippad paddy field in Kannur district of Kerala, India. With this new species, the genus is now represented by 16 species from Kerala, 21 from India and 126 valid species worldwide. A short note on Kaippad cultivation of paddy and new host records of genus *Antrocephalus* are provided.

Keywords: *Haltichellinae, new species, taxonomy, India.*

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Introduction

Antrocephalus is one of the most common genera of chalcid wasps one could encounter in any collection method and is distributed all across the inhabited parts of Asia, Europe, Australia and South America (Bouček, 1988). It is one of many confusing genera of chalcid family (Narendran, 1989; Sureshan, 1994) with its limits hard to define as it comes very close to *Hockeria* Walker and rarely to *Kriechbaumerella* Dalla Torre and only with extensive expertise in the taxonomy of these genera, one can authoritatively identify this genus (Narendran & van Achterberg, 2016). As a typical Haltichellinae member, sexual dimorphism is quite evident in the genus than the rest of chalcid counterparts with the flagellum of male thicker. One of the most peculiar diagnostic features of the genus is the long horse-shoe shaped pre-orbital carina running along the compound eye and joining behind the median ocellus along with an extended pronotal carina ending mostly as tubercles medially. Literatures and field observations attribute species of the genus as potential biocontrol agents against lepidopteran pests (Narendran, 1989; Narendran & van Achterberg, 2016). The latest revision of the genus from India was compiled by Narendran (1989). Sureshan (1994) added the latest valid species viz,

A. narendrani from Kasargod (Kerala).

Here we describe with illustrations a new species of *Antrocephalus* from Kaippad paddy fields in Kerala, India.

Materials and Methods

The new species was collected from a paddy field following the Kaippad cultivation in Keezhara (12°00'30.4" N, 75°19'21.7" E) in Kannur, Kerala, India by sweeping along the bunds of rice field and two meter inside from edges, swept once at each step in a figure of eight. The collected specimens were transferred to 70% ethyl alcohol, dried, card mounted and examined under LEICA M205 stereozoom microscope. The designated holotype of the new species was imaged using attached LEICA DFC2900 digital camera. Measurements were obtained using Leica LAS (Leica Application Suite V3.80) microsystems by Leica (Heerburgg, Switzerland). Images taken at varying focal planes were stacked using Leica Auto montage Software V3.80 and the final illustrations were post-processed for contrast and brightness using Adobe Photoshop CS5 (Version 12.0 x64) software. Further expeditions to collect additional samples did not materialize, and this description is based on the lone specimen collected and designated as holotype. The

specimen is currently deposited in entomological collections of Systematic Entomology Laboratory, Malabar Christian College and will be deposited in 'National Zoological Collections' of Zoological Survey of India, Western Ghats Regional Centre, Kozhikode (ZSIK).

Terms and abbreviations: Morphological terms used are adapted from Narendran (1989). The nomenclature for cuticular sculpturing follows Harris (1979). The general abbreviations of the terms are as follows unless noted otherwise; **fl_x**= Flagellum, x being the flagellum number; **mv**= Marginal vein; **OOL**= Ocello-Ocular line; **pmv**= Postmarginal vein; **POL**= Post ocellar line; **Gt_x**= Gastral terga, x being the tergum number.

Results

Genus *Antrocephalus* Kirby, 1883

Antrocephalus Kirby, 1883: 54, 63. Type species: *Halticella fascicornis* Walker; designated by Kirby (1883).

Narendran & van Achterberg (2016: 12) may be referred for complete list of synonymy.

Diagnosis: Head in frontal view with a horse shoe-shaped preorbital carina running beyond the median ocellus joining behind it; scrobe deep enclosing the median ocellus; pronotum with anterior carinae often indicated, forming submedian tubercles in most species; genotemporal margin often with a distinct genotemporal furrow; metasoma usually acuminate with two basal submedian short or long carinae on Gt₁.

Hosts: Pupae of Lepidoptera.

Distribution: Asia, Australia, Europe, and Oceania (New Guinea). Introduced in South America (Bouček, 1988).

Antrocephalus argentoflagellatus Binoy and Santhosh sp. n.

(Figs. 1–10)

[urn:lsid:zoobank.org:act:B7CFBF28-2244-448C-962C-7C487444B9CF](https://zoobank.org/act:B7CFBF28-2244-448C-962C-7C487444B9CF)

Material examined: Holotype ♀, INDIA: Kerala, Kannur district, Keezhara; Kaippad Paddy Field (12°00'30.4" N & 75°19'21.7" E), 24.vii.2017, Coll. K.M. Rajesh.

Diagnosis: In the key to Oriental species of *Antrocephalus* Kirby (Narendran, 1989), *A. argentoflagellatus* sp. n. keys near *A. japonicus* (Masi) in having Gt₁ more than half as long as abdomen, basal carinae more than 0.25× as Gt₁, scutellum slightly convex with posteriorly declining propodeum. However, it differs from *A. japonicus* in having 1) scutellum emarginate and bidentate with lateral margins emarginate, carinate (in *A. japonicus*, scutellum with lateral sides rounded, acarinate, apically emarginate); 2) scrobe not reaching the front ocellus and without traverse carina beneath front ocellus (in *A. japonicus*, scrobe reaching the front ocellus with traverse carina beneath front ocellus); 3) *pmv* 1.2× *mv* (in *A. japonicus*, *pmv* as long as *mv*); 4) metasoma distinctly longer than mesosoma (in *A. japonicus*, metasoma as long as mesosoma).

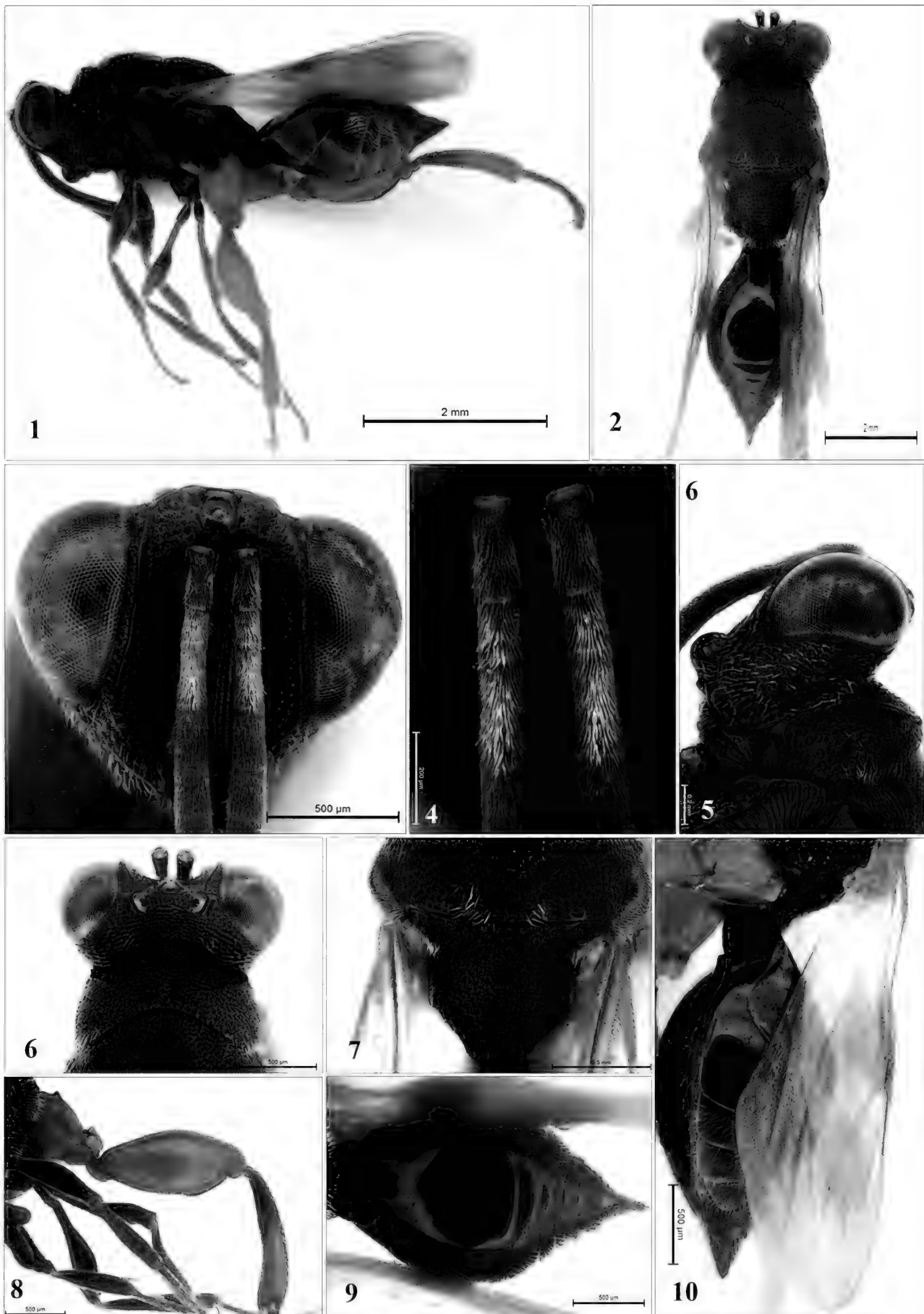
Description: Holotype ♀ (Figs. 1–10). Length 4.15 mm. Body black with the following parts as follows: eyes black; hind coxa and femur reddish brown, all tarsi testaceous.

Head: Head densely punctate, width in frontal view 1.3× its height (up to anterior clypeal margin) (Fig. 3); POL 1.3× OOL, with coarse setigerous punctae (Fig. 6); eyes glabrous, 1.9× as wide as long; strong horse-shoe shaped pre-orbital carina joining behind the median ocellus; genotemporal furrow distinct, deep; post orbital carina absent (Fig. 5); scape 7.0× as long as wide; fl₁ subquadrate, fl₂ 2.0× as long as wide, fl₂ and fl₃ with characteristic silver pubescence on the surface (Fig. 4).

Mesosoma: Pronotum dorsally with close pits and narrow interstices, anterior pronotal carina joining sub-medially forming weak tubercles (Fig. 6); axilla with yellowish brown patch of setae posteriorly; pits on mesoscutum and scutellum coarsely rugose punctate, with narrow interstices; scutellum 1.1× as long as wide, apically bidentate with lateral margins emarginate, deeply carinate (Fig. 7); lateral propodeal tooth distinct.

Wings: Fore wing infumated with dark infuscation joining *mv* and a faint one post *pmv*, veins deep brown, pubescence brown on lamina; *pmv* 1.2× *mv* (Fig. 10).

Legs: Hind coxa reddish brown with a prominent black tubercle dorsobasally; hind femur without inner ventro-basal tooth, 3.0× as long as wide, fairly pubescent on outer disc with short comb of serrulation restricted to



Figures 1–10. *Antrocephalus argentoflagellatus* Binoy and Santhosh **sp. n.** Holotype ♀ : **1.** Habitus, lateral view; **2.** Habitus, dorsal view; **3.** Head, frontal view; **4.** Flagellum 1–3, dorsal view; **5.** Head and pronotum, lateral view; **6.** Head and pronotum, dorsal view; **7.** Mesoscutum and scutellum, dorsal view; **8.** Hind leg; **9.** Metasoma, dorsal view; **10.** Fore wing and metasoma, lateral view.

apical third; hind tibia reddish brown with basal third brownish black (Fig. 8).

Metasoma: Metasoma longer than combined length of mesosoma; a short petiole visible laterally, dorsally inconspicuous; Gt_1 0.6× as long as metasoma, Gt_1 with basal carina more than 0.3× as long as Gt_1 (Fig. 9); Gt_6 rugose punctate with dense pubescence, syntergum with longitudinal carina (Fig. 10).

Male: Unknown.

Host: Unknown.

Distribution: India: Kerala.

Etymology: The species epithet in neuter gender is derived from the characteristic silver pubescence on flagellum 2-3.

Notes: Kaippad cultivation is an indigenous type of paddy cultivation followed in northern parts of Kerala wherein the medium for cultivation is saline in nature. Kaippad lands are specialized rice tracts traditionally developed from the wetland ecosystem of the areas which are swampy and waterlogged. They experience floods during the monsoons and salinity during summer owing to nearness to rivers that merge to the sea. The tidal currents from the nearby sea move through the river and enter Kaippad fields during the high tide and flow out during low tide. The river water is saline except during monsoon. Salt water from the sea enters the river during summer when the flow is low. When the water level in the river rises either due to tides or rain, the river water flows in to the paddy (Chandramohan, 2013). Even with this unique irrigation and variable nutrients available for development, paddy is susceptible to many pest species (Rajesh *et al.*, 2021). Many chalcid parasitoids are encountered in these fields and a better understanding of the taxonomic position of each of the parasitoid, will deem helpful in the natural control of the pest species, if the same goes above the economic threshold level of attack on paddy.

Members of *Antrocephalus* have been reported to attack a variety of lepidopteran hosts (Bouček, 1988) and lately two new host records are newly discovered. *Antrocephalus lugubris* (Masi, 1932) is newly recorded from the pupa of an unidentified *Lymantria* sp.

(Lymantriidae) and *A. sepyra* (Walker, 1846) is reported to attack the pupal stage of *Tagiades litigiosa* Möschler (Hesperiidae) from Kerala, India. The host-parasitoid status of the new species is presently unknown, but once established the same can be used to initiate biocontrol mechanisms of the pest species that it parasitizes on.

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First Record of *Camponotus japonicus* Mayr, 1866 (Hymenoptera: Formicidae) from India

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Abstract

Camponotus japonicus Mayr, 1866 is recorded for the first time from India. Formerly, it was documented from China, Japan, Mongolia, North Korea, Pakistan, Philippines, Russia and South Korea.

Keywords: New record, Formicinae, India.

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Introduction

The *Camponotus* Mayr, 1861 is a diverse genus which belongs to subfamily Formicinae. The genus has a wide distribution and occupies almost all biogeographical regions of the World (Bolton *et al.*, 2007). At present 1,053 valid species and 443 subspecies are described from all over the world (Bolton, 2021). From India the genus is represented by 75 species (Bharti *et al.*, 2016).

The significant contributions to the taxonomy of genus *Camponotus* from all over the globe include Mayr (1861), Emery (1896, 1920, 1925), Forel (1912, 1914), Arnold (1922), Wheeler (1922) and Santschi (1926, 1928). Whereas, Blaimer *et al.* (2015) and Ward *et al.* (2016) revised the classification of the genus on the basis of molecular phylogeny.

From India, taxonomic contributions include Bingham (1903), Datta & Raychaudhuri (1985), Karmaly & Narendran (2006), Bharti & Wachkoo (2014, 2015), Wachkoo (2015), Bharti *et al.* (2016), Wachkoo & Akbar (2016).

Camponotus japonicus was described by Mayr, 1866 from Japan. It has been classified as subspecies of *Camponotus pennsylvanicus* (Forel, 1879, 1904; Ruzsky, 1905) and subspecies of *Camponotus herculeanus* (Emery, 1908; Wheeler, 1906, 1909, 1921; Ruzsky, 1925; Kuznetsov-Ugamsky, 1929; Yasumatsu & Brown, 1951). Later, it was raised to species level (Bingham, 1903; Santschi, 1920, 1925;

Emery, 1925; Ruzsky, 1926; Wheeler, 1927, 1928; Karavaiev, 1929; Stitz, 1934; Yasumatsu & Brown, 1957; Arnol'di, 1967; Kupyanskaya, 1990). The species is classified as senior synonym of *Camponotus japonicus miltotus*, *Camponotus sanguinea* and *Camponotus japonicus wui* by Yasumatsu & Brown (1951). Radchenko (1997) sited it as senior synonym of *Camponotus aterrimus* (and its junior synonym *Camponotus japonicus manczshuricus*).

Mostly the colonies of *C. japonicus* are monogynous, but in some cases large colonies are found to be polygynous (Wang *et al.*, 1991). The workers prey upon small arthropods and honey dew collected from aphids (Wu & Wang, 1995). Abe (1973) mentioned that the species nests in soil in open habitats and nest entrance's open directly without a surrounding mound.

This species displays a symbiotic association with myrmecophilous *Lycaenid argyrognomon*. The caterpillars of *Lycaenid argyrognomon* pupate within the nests of *C. japonicus* in the field to protect themselves from predators (Watanabe & Hagiwara 2009 ; Mizuno *et al.*, 2018).

During the present study, we redescribed *Camponotus japonicus* Mayr, 1866 from India complemented with digital images.

Materials and Methods

Taxonomic and morphometric analysis

was conducted using a Nikon SMZ 1500 stereo zoom microscope. Digital images of the specimens were captured using a Nikon SMZ 1500 stereomicroscope fitted with an MP (Micro Publisher) digital camera and Auto Montage (Syncroscopy, a division of synoptics Ltd.) software. All the images were cleaned with Adobe Photoshop CS5 and Helicon Filter 5. Morphological terminology and standard measurements follow Salata *et al.* (2020). Specimens examined are deposited at “Punjabi University Patiala Ant Collection” (PUAC) at Department of Zoology and Environmental Sciences, Punjabi University, Patiala, Punjab, India.

Abbreviations used

HL: head length; measured in a straight line from mid-point of anterior clypeal margin to mid-point of posterior margin of head in full-face view;

HW: head width; measured in full-face view directly above the eyes;

SL: scape length; maximum straight-line length of scape excluding the basal condylar bulb;

PW: pronotum width; maximum width of pronotum in dorsal view;

PRL: propodeum length; measured in lateral view, from metanotal suture to posterior-most point of propodeum;

PRW: propodeal width; maximum width of propodeum in dorsal view;

PTH: petiole height; the chord of ventral petiolar profile at node level is the reference line perpendicular to which the maximum height of petiole is measured in lateral view;

PTW: petiole width; maximum width of the petiolar node in lateral view;

WL: Weber's length; measured as diagonal length from the anterior end of the neck shield to the posterior margin of the propodeal lobe.

Ratios

CI: cephalic ratio, HL/HW;

SI: scape ratio, SL/HL;

PI: petiole ratio, PTH/PTW.

Result

Material examined: INDIA, Arunachal Pradesh, Tawang [27.5866°N, 91.8582°E], 1700

m, Hand picking, 08.ix.2019, 3w. Tarun Dhadwal leg. (PUAC).

Description of Worker (Figs: 1-6):

Measurements (in mm):

Major Worker: HL: 2.34-2.58, HW: 2.13-2.40, SL: 2.28-2.38, WL: 2.07-2.17, PW: 1.35-1.38, PRL: 0.63-0.65, PRW: 0.39-0.43, PTH: 0.66-1.01, PTW: 0.60-0.80, CI: 1.09-1.07, SI: 0.97-0.92, PI: 1.11-1.26 (n = 2).

Minor Worker: HL: 1.74, HW: 1.53, SL: 1.77, WL: 2.4, PW: 1.29, PRL: 0.57, PRW: 0.3, PTH: 0.72, PTW: 0.54, CI: 1.13, SI: 1.01, PI: 1.33 (n=1).

In major worker, head is longer than broad, with slightly convex lateral margins and an emarginated posterior margin. Scape long, surpasses the posterior border of head by more than a quarter of its length. Anterior margin of clypeus is convex rather than carinate. Eyes are small and prominently situated before the midlength of the head. Mandibles are short and have 6 teeth. While in the case of minor worker, head is relatively small and sub-rectangular in shape. Mandibles have 5 teeth.

Head in both major and minor worker opaque, densely microreticulate punctate. Mandibles are gleaming and punctured. Major workers have more hair on their bodies than minor workers. Short erect hair covers the posterior margin of the head. The anterior border of the clypeus is covered with long erect setae.

Mesosoma of both major and minor workers is not consistently convex, with a noticeable suture between the pronotum and the mesonotum. The mesosoma of a major worker is strong and obliquely truncate at the propodeum. In minor workers, the slope of the metanotum is less steep. Propodeal spiracle elongate. The whole surface of the mesosoma is covered in long erect and sub erect hair. Petiole node in Major worker is quite thick, anteriorly convex, and posteriorly flat. Petiole having 2 or 3 standing hair on dorsal surface.

Gaster is swathed in long golden recumbent hair. The hindtibia is prismatic, having 9 to 12 spines on the inner side of the tibia.

Body coloration: Both major and minor workers are black in colour.

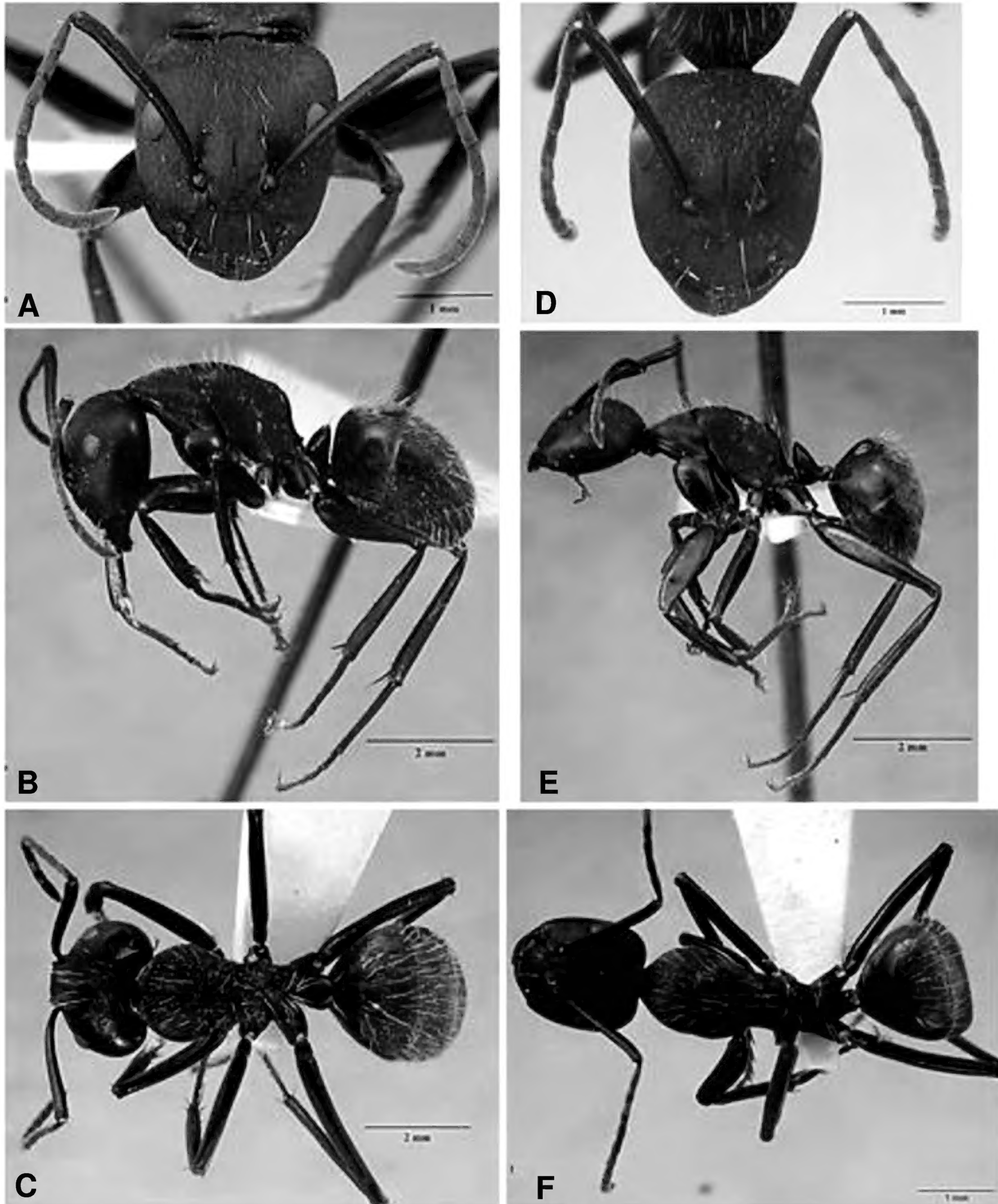


Figure 1: *Camponotus japonicus* Mayr, 1866: **A:** Major worker Head, full face view; **B:** Major worker Body, lateral view; **C:** Major worker Body, dorsal view; **D:** Minor worker Head, full face view; **E:** Minor worker Body, lateral view; **F:** Minor worker Body, dorsal view.

Distribution: The species is widely distributed in China, Japan, Mongolia, North Korea, Pakistan, Philippines, Russia and South Korea.

Bionomics: The workers were collected by hand picking method over the grass near Kitpi lake from Tawang district of Arunachal Pradesh. The

lake is situated at an elevation of 1700 meters, with an average daily temperature of 20°C.

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